rings. Preferred heterocyclic radicals include unsaturated heterocyclic radicals such as furanyl, thiophenyl, pyrrolyl, pyrazolyl, imidazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2-dithiolyl, 1,3-dithiolyl, 1,2,3-oxathiolyl, isoxazolyl, oxazolyl, thiazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,3,4-oxatriazolyl, 1,2,3,5-oxatriazolyl, 1,2,3-dioxazolyl, 1,2,4-dioxazolyl, 1,3,2-dioxazolyl, 1,3,4-dioxazolyl, 1,2,5-oxathiazolyl, 1,3-oxathiolyl, 1,2-pyranyl, 1,4-pyranyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,2,4-triazinyl, 1,2,3-triazinyl, 1,2,4-oxazinyl, 1,3,2-oxazinyl, 1,2,6-oxazinyl, 1,4-oxazinyl, isoxazinyl, 1,2,5-oxathiazinyl, 1,4-oxazinyl, o-isoxazinyl, p-isoxazinyl, 1,2,5-oxathiazinyl, 1,2,6-oxathiazinyl, 1,3,5,2-oxadiazinyl, azepinyl, oxepinyl, thiepinyl, 1,2,4-diazepinyl, benzofuranyl, isobenzofuranyl, thionaphthenyl, indolyl, indolenyl, 2-isobenzazolyl, 1,5-pyrindinyl, pyrano[3,4-b]pyrrolyl, benzpyrazolyl, benzisoxazolyl, benzoxazolyl, anthranilyl, 1,2-benzopyranyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, naphthyridinyl, pyrido[3,4-b]pyridinyl, pyrido[4,3-b]pyridinyl, pyrido[2,3-b]pyridinyl, 1,3,2-benzozazinyl, 1,4,2-benzoxazinyl, 2,3,1-benzoxazinyl, 3,1,4-benzoxazinyl, 1,2-benzisoxazinyl, 1,4-benzisoxazinyl, carbazolyl, purinyl, and partially saturated heterocyclic radicals selected from the list above. All of the preferred heterocyclic radicals contain at least one double bond. When the heterocyclic radical is partially saturated, one or more of the olefins in the unsaturated ring system is saturated; the partially saturated heterocyclic radical still contains at least one double bond. It is more preferred that hetaryl is pyridinyl.

Of the compounds of this invention preferred members are those in which R^1 is hydrogen.

Compounds of this invention having the amidino carbamate moiety at the 42- or 31,42-positions can be prepared by conversion of rapamycin to the 4-nitrophenylcarbonate (as illustrated in Example 1), followed by reaction with an appropriately functionalized amidine. Mixtures of 42- and 31,42-carbamates can be separated by chromatography.

The 31-amidino carbamates of this invention can be prepared by protecting the 42-alcohol of rapamycin with a protecting group, such as with a tert-butyl dimethylsilyl group, followed by carbamylation of the, 31-position by the procedures described above. The preparation of rapamycin 42-silyl ethers is described in U.S. Pat. No. B1 5,120,842, which is hereby incorporated by reference. Removal of the protecting group provides the 31-esterified compounds. In the case of the tert-butyl dimethylsilyl protecting group, deprotection can be accomplished under mildly acidic conditions, such as acetic acid/water/THF. The deprotection procedure is described in Example 15 of U.S. Pat. No. 5,118,678, which is hereby incorporated by reference.

Having the 31-position carbamylated and the 42-position deprotected, the 42-position can be carbamylated using a different amidine agent than was reacted with the 31-carbonate, to give compounds having different amidino carbamates at the 31- and 42- positions. Alternatively, the 42-carbamylated compounds, prepared as described above, can be converted to the 31-carbonate-42-carbamate and reacted with a different amidine to provide compounds having different carbamates at the 31- and 42-positions.

This invention also covers analogous carbamates of other rapamycins such as, but not limited to, 29-demethoxyrapamycin, [U.S. Pat. No. 4,375,464, 32-demethoxyrapamycin under C.A. nomenclature]; rapamycin derivatives in which the double bonds in the 1-, 3-, and/or 5-positions have been reduced [U.S. Pat. No. 5,023,262]; 29-desmethylrapamycin [U.S. Pat. No. 5,093,339, 32-desmethylrapamycin under C.A. nomenclature]; 7,29-bisdesmethylrapamycin [U.S. Pat. No. 5,093,338, 7,32-desmethylrapamycin under C.A. nomenclature]; and 15-hydroxyrapamycin [U.S. Pat. No. 5,102,876]. The disclosures in the above cited U.S. Patents are hereby incorporated by reference.

Immunosuppressive activity for representative compounds of this invention was evaluated in an in vitro standard pharmacological test procedure to measure the inhibition of lymphocyte proliferation (LAF) and in two in vivo standard pharmacological test procedures. The pinch skin graft test procedure measures the immunosuppressive activity of the compound tested as well as the ability of the compound tested to inhibit or treat transplant rejection. The adjuvant arthritis standard pharmacological test procedure, which measures the ability of the compound tested to inhibit immune mediated inflammation. The adjuvant arthritis test procedure is a standard pharmacological test procedure for rheumatoid arthritis. The procedures for these standard pharmacological test procedures are provided below.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated radioactivity is determined. Inhibition of lymphoproliferation is assessed as percent change in counts per minute from non-drug treated controls. For each compound evaluated, rapamycin was also evaluated for the purpose of comparison. An IC_{50} was obtained for each test compound as well as for rapamycin. When evaluated as a comparator for the representative compounds of this invention, rapamycin had an IC_{50} ranging from 0.4–1.0 nM. The results obtained are provided as an IC_{50} and as the percent inhibition of T-cell proliferation at 0.1 μM. The results obtained for the representative compounds of this invention were also expressed as a ratio compared with rapamycin. A positive ratio indicates immunosuppressive activity. A ratio of greater than 1 indicates that the test compound inhibited thymocyte proliferation to a greater extent than rapamycin. Calculation of the ratio is shown below.

$$\frac{IC_{50} \text{ of Rapamycin}}{IC_{50} \text{ of Test Compound}}$$

Representative compounds of this invention were also evaluated in an in vivo test procedure designed to determine the survival time of pinch skin graft from male BALB/c donors transplanted to male C_3H(H-2K) recipients. The method is adapted from Billingham R. E. and Medawar P. B., J. Exp. Biol. 28:385–402, (1951). Briefly, a pinch skin graft from the donor was grafted on the dorsum of the recipient as a allograft, and an isograft was used as control in the same region. The recipients were treated with either varying concentrations of test compounds intraperitoneally or orally. Rapamycin was used as a test control. Untreated recipients serve as rejection control. The graft was monitored daily and observations were recorded until the graft became dry and formed a blackened scab. This was considered as the rejection day. The mean graft survival time (number of days ±S.D.) of the drug treatment group was compared with the control group. The following table shows the results that were obtained. Results are expressed as the mean survival time in days. Untreated (control) pinch skin grafts are usually rejected within 6–7 days. Compounds were tested using a dose of 4 mg/kg.

The adjuvant arthritis standard pharmacological test procedure measures the ability of test compounds to prevent immune mediated inflammation and inhibit or treat rheumatoid arthritis. The following briefly describes the test procedure used. A group of rats (male inbread Wistar Lewis rats) are pre-treated with the compound to be tested (1 h prior to antigen) and then injected with Freud's Complete Adjuvant (FCA) in the right hind paw to induce arthritis. The rats are then orally dosed on a Monday, Wednesday, Friday schedule from day 0–14 for a total of 7 doses. Both hind paws are measured on days 16, 23, and 30. The difference in paw volume (mL) from day 16 to day 0 is determined and a percent change from control is obtained. The left hind paw (uninjected paw) inflammation is caused by T-cell mediated inflammation and is recorded in the above table (% change from control). The right hind paw inflammation, on the other hand, is caused by nonspecific inflammation. Compounds were tested at a dose of 5 mg/kg. The results are expressed as the percent change in the uninjected paw at day 16 versus control; the more negative the percent change, the more potent the compound. Rapamycin provided –90% change versus control, indicating that rapamycin treated rats had 90% less immune induced inflammation than control rats.

The results obtained in these standard pharmacological test procedures are provided following the procedure for making the specific compounds that were tested.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this invention. The results obtained in the LAF test procedure indicates suppression of T-cell proliferation, thereby demonstrating the immunosuppressive activity of the compounds of this invention. Further demonstration of the utility of the compounds of this invention as immunosuppressive agents was shown by the results obtained in the skin graft and adjuvant arthritis standard pharmacological test procedures. Additionally, the results obtained in the skin graft test procedure further demonstrates the ability of the compounds of this invention to treat or inhibit transplantation rejection. The results obtained in the adjuvant arthritis standard pharmacological test procedure further demonstrate the ability of the compounds of this invention to treat or inhibit rheumatoid arthritis.

Based on the results of these standard pharmacological test procedures, the compounds are useful in the treatment or inhibition of transplantation rejection such as kidney, heart, liver, lung, bone marrow, pancreas (islet cells), cornea, small bowel, and skin allografts, and heart valve xenografts; in the treatment or inhibition of graft vs. host disease; in the treatment or inhibition of autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; and diseases of inflammation such as psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease, pulmonary inflammation (including asthma, chronic obstructive pulmonary disease, emphysema, acute respiratory distress syndrome, bronchitis, and the like), and eye uveitis.

Because of the activity profile obtained, the compounds of this invention also are considered to have antitumor, antifungal activities, and antiproliferative activities. The compounds of this invention therefore also useful in treating solid tumors, adult T-cell leukemia/lymphoma, fungal infections, and hyperproliferative vascular diseases such as restenosis and atherosclerosis. When used for restenosis, it is preferred that the compounds of this invention are used to treat restenosis that occurs following an angioplasty procedure. When used for this purpose, the compounds of this invention can be administered prior to the procedure, during the procedure, subsequent to the procedure, or any combination of the above.

When administered for the treatment or inhibition of the above disease states, the compounds of this invention can be administered to a mammal orally, parenterally, intranasally, intrabronchially, transdermally, topically, intravaginally, or rectally.

It is contemplated that when the compounds of this invention are used as an immunosuppressive or antiinflammatory agent, they can be administered in conjunction with one or more other immunoregulatory agents. Such other immunoregulatory agents include, but are not limited to azathioprine, corticosteroids, such as prednisone and methylprednisolone, cyclophosphamide, rapamycin, cyclosporin A, FK-506, OKT-3, and ATG. By combining the compounds of this invention with such other drugs or agents for inducing immunosuppression or treating inflammatory conditions, the lesser amounts of each of the agents are required to achieve the desired effect. The basis for such combination therapy was established by Stepkowski whose results showed that the use of a combination of rapamycin and cyclosporin A at subtherapeutic doses significantly prolonged heart allograft survival time. [Transplantation Proc. 23: 507 (1991)].

The compounds of this invention can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid. When formulated orally, it has been found that 0.01% Tween 80 in PHOSAL PG-50 (phospholipid concentrate with 1,2-propylene glycol, A. Nattermann & Cie. GmbH) provides an acceptable oral formulation.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, lethicins, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which am sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

The compounds of this invention may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. The compounds of this invention may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood sire am via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semi-solid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound which may be administered to a fungally affected area.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected dally dosages of active compound would be 0.1 μg/kg–100 mg/kg, preferably between 0.001–25 mg/kg, and more preferably between 0.01–5 mg/kg. Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, nasal, or intrabronchial administration will be determined by the administering physician based on experience with the individual subject treated. Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is subdivided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form.

The following examples illustrate the preparation and biological activities of representative compounds of this invention.

EXAMPLE 1

42-O-(4-Nitro-phenoxycarbonyl)rapmycin

To a solution of 5.15 g (5.633 mmol) of rapamycin in 40 ml of methylene chloride cooled to −78° C. with dry ice/acetone bath, was added 0.7 ml dry pyridine and 1.70 g (8.450 mmol) of p-nitrophenylchloroformate dissolved in 10 ml methylene chloride. The reaction mixture was allowed to warm to ambient and stirred overnight under nitrogen. The reaction mixture was concentrated in vacuo and partitioned between ether and water. The organic phase was washed with 0.1N HCl (3×), then with a saturated sodium chloride solution (2×), dried over magnesium sulfate, filtered and concentrated under vacuum to give a pale yellow solid. Purification by flash column chromatography (elution with 40% then 50% ethyl acetate/hexanes) gave 5.41 g (88%) of the title compound as a pale yellow solid.

^{1}H NMR (DMSO) δ 8.3 and 7.5 (d and d, aromatic-H, 4H), 4.5 (m, 42C-H, 1H).

MS (–) FAB m/z: 1078 (M^-), 590 (Southern Fragment).

EXAMPLE 2

Rapamycin 42-ester with (imino-phenyl-methyl)carbamic acid

To a solution of 1.0024 g (0.9287 mmol) of 42-O-(4-Nitrophenoxycarbonyl)rapamycin in 5 ml of DMF was added 0.2231 g (1.8574 mmol) of benzamidine. The reaction mixture was allowed to stir under nitrogen for 2 hours at ambient temperature, then was diluted with ethyl acetate and washed with portions of H_2O and brine. The organic phase was dried over magnesium sulfate, filtered and concentrated under vacuum to yield crude product. Purification by flash column chromatography (elution with 60% then 80% ethyl acetate/hexanes) gave 0.1032 g (10%) of the title compound as a pale yellow solid.

^{1}H NMR (DMSO) δ 9.05 (m, N-H, 2H), 7.98–7.48 (m, aromatic-H, 5H), 4.48 (m, 42C-H, 1H).

MS (–) FAB m/z: 1059 (M^-), 590 (Southern Fragment), 467 (Northern Fragment).

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 2.05 nM

LAF ratio: 0.47

Skin graft survival: 9.8±0.8

Percent change in adjuvant arthritis versus control: –91%

EXAMPLE 3

Rapamycin 42-ester with (imino-pyridin-2-yl)methyl)carbamic acid

To 0.585 g (3.7 mmol) of 2-amidinopyridine hydrochloride was added one equivalent of 0.1M sodium hydroxide/methanol after which the solvent was removed in vacuo. To the solution of the free base in 20 ml of DMF was added 4.0 g (3.7 mmol) of 42-O-(4-Nitro-phenoxycarbonyl)rapamycin. The reaction mixture was allowed to stir under nitrogen for 6 hours at ambient temperature, then was diluted with ethyl acetate and washed with portions of H_2O and brine. The organic phase was dried over magnesium sulfate, filtered and concentrated under vacuum to yield crude product. Purification by flash column chromatography (elution with 1:1 ethyl acetate: hexanes, then 100% ethyl acetate) gave 0.47 g (12%) of the title compound as an off-white solid.

^{1}H NMR (DMSO) δ 9.05 (m, N-H, 2H), 8.68–8.72 (m, Ar-H, 1H), 8.28–8.24 (m, Ar-H, 1H), 8.0–7.94 (m, Ar-H, 1H), 7.66–7.62 (m, Ar-H, 1H), 4.4 (m, 42C-H, 1H).

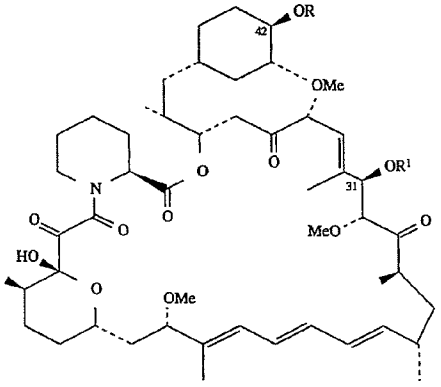
MS (–) FAB m/z: 1060 (M^-), 590 (Southern Fragment), 468 (Northern Fragment).

Results obtained in standard pharmacological rest procedures:

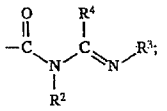
LAF IC_{50}: 0.60 and 0.55 nM

LAF ratio: 1.50 and 0.78
Skin graft survival: 11.7±1.0
What is claimed is:

1. A method of treating transplantation rejection graft vs. host disease in a mammal in need thereof, which comprises administering to said mammal an antirejection effective amount of a compound of the structure



wherein R and R^1 are each, independently, hydrogen, or



R^2 and R^3 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—CO_2R^5$, —COR^5, —CN, $—NO_2$, $—SO_2R^5$, $—SO_3R^5$, $—OR^5$, $—SR^5$, or Ar;

R^4 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—CF_3$, $—NR^5R^6$, $—CO_2R^5$, —COR^5, $CONR^5R^6$, $—NO_2$, halogen, $—OR^5$, $—SR^5$, —CN, $—SO_2R^5$, $—SO_3R^5$, $—SO_2NR^5R^6$, or Ar;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or Ar;

Ar is phenyl, naphthyl, or hetaryl, wherein the foregoing may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, $—SO_3H$, and $—CO_2H$;

hetaryl is a heterocyclic radical selected from the group consisting of furanyl, thiophenyl, pyrrolyl, pyrazolyl, imidazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2-dithiolyl, 1,3-dithiolyl, 1,2,3-oxathiolyl, isoxazolyl, oxazolyl, thiazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,3,4-oxatriazolyl, 1,2,3,5-oxatriazolyl, 1,2,3-dioxazolyl, 1,2,4-dioxazolyl, 1,3,2-dioxazolyl, 1,3,4-dioxazolyl, 1,2,5-oxathiazolyl, 1,3-oxathiolyl, 1,2-pyranyl, 1,4-pyranyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,2,4-triazinyl, 1,2,3-triazinyl, 1,2,4-oxazinyl, 1,3,2-oxazinyl, 1,2,6-oxazinyl, 1,4-oxazinyl, isoxazinyl, 1,2,5-oxathiazinyl, 1,4-oxazinyl, o-isoxazinyl, p-isoxazinyl, 1,2,5-oxathiazinyl, 1,2,6-oxathiazinyl, 1,3,5,2-oxadiazinyl, azepinyl, oxepinyl, thiepinyl, 1,2,4-diazepinyl, benzofuranyl, isobenzofuranyl, thionaphthenyl, indolyl, indolenyl, 2-isobenzazolyl, 1,5-pyrindinyl, pyrano[3,4-b]pyrrolyl, benzpyrazolyl, benzisoxazolyl, benzoxazolyl, anthranilyl, 1,2-benzopyranyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, naphthyridinyl, pyrido[3,4-b]pyridinyl, pyrido[4,3-b]pyridinyl, pyrido[2,3-b]pyridinyl, 1,3,2-benzoxazinyl, 1,4,2-benzoxazinyl, 2,3,1-benzoxazinyl, 3,1,4-benzoxazinyl, 1,2-benzisoxazinyl, 1,4-benzisoxazinyl, carbazolyl, and purinyl;

with the proviso that R and R^1 are both not hydrogen, or a pharmaceutically acceptable salt thereof.

* * * * *

US005516781A

United States Patent [19]

Morris et al.

[11] **Patent Number: 5,516,781**

[45] **Date of Patent: *May 14, 1996**

[54] **METHOD OF TREATING RESTENOSIS WITH RAPAMYCIN**

[75] Inventors: **Randall E. Morris**, Los Altos; **Clare R. Gregory**, Menlo Park, both of Calif.

[73] Assignee: **American Home Products Corporation**, Madison, N.J.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,288,711.

[21] Appl. No.: **238,305**

[22] Filed: **May 12, 1994**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 980,000, Nov. 23, 1992, abandoned, which is a continuation of Ser. No. 819,314, Jan. 9, 1992, abandoned.

[51] **Int. Cl.**[6] **A61K 31/345**

[52] **U.S. Cl.** **514/291**

[58] **Field of Search** 514/291, 56; 424/122

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,929,992	12/1975	Sehgal et al.	514/291
4,885,171	12/1989	Surendra et al.	424/122
5,078,999	1/1992	Warner et al.	424/122
5,080,899	1/1992	Sturm et al.	424/122
5,100,899	3/1992	Calne	514/122
5,252,579	10/1993	Skotnicki et al.	514/291
5,256,790	10/1993	Nelson	514/291
5,288,711	2/1994	Mitchell et al.	514/56

FOREIGN PATENT DOCUMENTS

0401747	6/1990	European Pat. Off. .

OTHER PUBLICATIONS

Akselband, Y., Transplantation Proc., 23:2833 (1991).
Woo, J., Cytokine, 3: 472 (1991).
Stepkowski, S., Transplantation Proc., 23: 507 (1991).
Cramer, D., Transplantation, 50: 554 (1990).
Kay, J., Immunology, 72: 544 (1991).
Ferns, G., Am. J. Path., 137: 403 (1990).
Gregory C. R., et al., American Society of Transplant Physicians, 13th Annual Meeting, May 16–18, 1994; Abstract (38) 9, mailed to attendees Apr. 27, 1994.
Gregory C. R., et al., American Society of Transplant Physicians, 13th Annual Meeting, May 16–18, 1994; Abstract (38) 10, mailed to attendees Apr. 27, 1994.
Gregory C. R., et al., Transplantation Proceedings, 25:1, 120–121 (Feb. 1993).
Gregory C. R., et al., Transplantation Proceedings, 25:1, 770–771 (Feb. 1993).
Gregory C. R., et al., Transplantation, 55:1409–1418 (Jun. 1993).
Gregory C. R., et al., Veterinary Surgery, Abstract 11:40 (Jan. 1993).
Gregory C. R. et al., J. Heart and Lung Transplantation, Abstract 27, 11:197 (1992).
Gregory C. R. et al., FASEB, Abstract 21: 6:A940 (1992).
Meiser, B. M. et al., J. Heart and Lung Tranplantation, Abstract 3:9–55 (1990).
Meiser, B. M. et al., Lancet 338:1297–1298 (1991).
Ferns, G. A. et al., Circulation, Abstract 0727, 80 (Supp. II) 183 (1989).
Jonasson, L. et al., Proc. Natl. Acad. Sci., 85:2303–2306 (Apr. 1988).
Morris, P. J. et al., Transplantation Reviews, 6:39 (1992).
Staruch, M. J., FASEB 3:3411 (1989).
Martel, R. R., Canadian Journal of Physiol. Pharmacology, 55:48 (1977).
Morris, R. E., Med. Sci. Res. 17:877 (1989).
Baeder, W. L., Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract) (1990).
Eng, C. P. et al., The Journal of Antiboitics, 37:1231 (1984).
The Merck Index, 9th ed., 6152:820–821 (1976).
Weaver, J. L., J. Cell Biology, Abstract 1308, 111:(5 Part 2)234a.

Primary Examiner—Michael G. Wityshyn
Assistant Examiner—Jean C. Witz
Attorney, Agent, or Firm—Arnold S. Milowsky

[57] **ABSTRACT**

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of rapamycin alone or in combination with mycophenolic acid.

5 Claims, No Drawings

METHOD OF TREATING RESTENOSIS WITH RAPAMYCIN

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of U.S. patent application Ser. No. 07/980,000, filed Nov. 23, 1992, now abandoned, which is a continuation of U.S. patent application Ser. No. 07/819,314, filed Jan. 9, 1992, now abandoned.

BACKGROUND OF THE INVENTION

Many individuals suffer from heart disease caused by a partial blockage of the blood vessels that supply the heart with nutrients. More severe blockage of blood vessels in such individuals often leads to hypertension, ischemic injury, stroke, or myocardial infarction. Typically vascular occlusion is preceded by vascular stenosis resulting from intimal smooth muscle cell hyperplasia. The underlying cause of the intimal smooth muscle cell hyperplasia is vascular smooth muscle injury and disruption of the integrity of the endothelial lining. The overall disease process can be termed a hyperproliferative vascular disease because of the etiology of the disease process. Intimal thickening following arterial injury can be divided into three sequential steps: 1) initiation of smooth muscle cell proliferation following vascular injury, 2) smooth muscle cell migration to the intima, and 3) further proliferation of smooth muscle cells in the intima with deposition of matrix. Investigations of the pathogenesis of intimal thickening have shown that, following arterial injury, platelets, endothelial cells, macrophages and smooth muscle cells release paracrine and autocrine growth factors (such as platelet derived growth factor, epidermal growth factor, insulin-like growth factor, and transforming growth factor) and cytokines that result in the smooth muscle cell proliferation and migration. T-cells and macrophages also migrate into the neointima. [Haudenschild, C., *Lab. Invest,* 41:407 (1979); Clowes, A., *Circ. Res.* 56:139 (1985); Clowes, A., J, *Cardiovas. Pharm.* 14 (Suppl. 6): S12 (1989); Manderson, J., Arterio. 9:289 (1989); Forrester, J., *J. Am. Coil. Cardiol.* 17:758 (1991)]. This cascade of events is not limited to arterial injury, but also occurs following injury to veins and arterioles.

Vascular injury causing intimal thickening can be broadly categorized as being either biologically or mechanically induced. Artherosclerosis is one of the most commonly occurring forms of biologically mediated vascular injury leading to stenosis. The migration and proliferation of vascular smooth muscle plays a crucial role in the pathogenisis of artherosclerosis. Artherosclerotic lesions include massive accumulation of lipid laden "foam cells" derived from monocyte/macrophage and smooth muscle cells. Formation of "foam cell" regions is associated with a breech of endothelial integrity and basal lamina destruction. Triggered by these events, restenosis is produced by a rapid and selective proliferation of vascular smooth muscle cells with increased new basal lamina (extracellular matrix) formation and results in eventual blocking of arterial pathways. [Davies, P. F., *Artherosclerosis Lab. Invest.* 55:5 (1986)].

Mechanical injuries leading to intimal thickening result following balloon angioplasty, vascular surgery, transplantation surgery, and other similar invasive processes that disrupt vascular integrity. Intimal thickening following balloon catheter injury has been studied in animals as a model for arterial restenosis that occurs in human patients following balloon angioplasty. Clowes, Ferns, Reidy and others

have shown that deendothelilization with an intraarterial catheter that dilates an artery injures the innermost layers of medial smooth muscle and may even kill some of the innermost cells. [Schwartz, S. M., *Human Pathology* 18:240 (1987); Fingerle, J., *Ateriosclerosis* 10:1082 (1990)]. Injury is followed by a proliferation of the medial smooth muscle cells, after which many of them migrate into the intima through fenestrae in the internal elastic lamina and proliferate to form a neointimal lesion.

Vascular stenosis can be detected and evaluated using angiographic or sonographic imaging techniques [Evans, R. G., *JAMA* 265:2382 (1991)] and is often treated by percutaneous transluminal coronary angioplasty (balloon catheterization). Within a few months following angioplasty, however, the blood flow is reduced in approximately 30–40 percent of these patients as a result of restenosis caused by a response to mechanical vascular injury suffered during the angioplasty procedure, as described above. [Pepine, C., *Circulation* 81:1753 (1990); Hardoff, R., *J. Am. Coll. Cardiol.* 15 1486 (1990)].

In an attempt to prevent restenosis or reduce intimal smooth muscle cell proliferation following angioplasty, numerous pharmaceutical agents have been employed clinically, concurrent with or following angioplasty. Most pharmaceutical agents employed in an attempt to prevent or reduce the extent of restenosis have been unsuccessful. The following list identifies several of the agents for which favorable clinical results have been reported: lovastatin [Sahni, R., *Circulation* 80 (Suppl.) 65 (1989); Gellman, J., *J. Am. Coll. Cardiol.* 17:251 (1991)]; thromboxane A_2 synthetase inhibitors such as DP-1904 [Yabe, Y., *Circulation* 80 (Suppl.) 260 (1989)]; eicosapentanoic acid [Nye, E., *Aust. N.Z. J. Med.* 20:549 (1990)]; ciprostene (a prostacyclin analog) [Demke, D., *Brit. J. Haematol* 76 (Suppl.): 20 (1990); Darius, H., *Eur. Heart J.* 12 (Suppl.): 26 (1991)]; trapidil (a platelet derived growth factor) [Okamoto, S., *Circulation* 82 (Suppl.): 428 (1990)]; angiotensin convening enzyme inhibitors [Gottlieb, N., *J. Am. Coll. Cardiol.* 17 (Suppl. A): 181A (1991)]; and low molecular weight heparin [de Vries, C., *Eur. Heart J.* 12 (Suppl.): 386 (1991)].

In an attempt to develop better agents for preventing or reducing smooth muscle proliferation and intimal thickening, the use of balloon catheter induced arterial injury in a variety of mammals has been developed as a standard model of vascular injury that will lead to intimal thickening and eventual vascular narrowing. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab, Invest,* 41:407 (1979); Clowes, A. W., *Lab. Invest.* 49:208 (1983); Clowes, A. W., *J. Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. Many compounds have been evaluated in this standard animal model. The immunosuppressive agent cyclosporin A has been evaluated and has produced conflicting results. Jonasson reported that cyclosporin A caused an inhibition of the intimal proliferative lesion following arterial balloon catheterization in vivo, but did not inhibit smooth muscle cell proliferation in vitro. [Jonasson, L., *Proc. Natl. Acad. Sci.* 85:2303 (1988)]. Ferns, however reported that when de-endothelilized rabbits were treated with cyclosporin A, no significant reduction of intimal proliferation was observed in vivo. Additionally, intimal accumulations of foamy macrophages, together with a number of vacuolated smooth muscle cells in the region adjacent to the internal elastic lamina were observed, indicating that cyclosporin A may modify and enhance lesions that form at the sites of arterial injury. [Ferns, G. A., *Circulation* 80 (Supp): 184 (1989); Ferns, G., *Am. J. Path.* 137:403 (1990)].

Rapamycin, a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus* [U.S. Pat. No. 3,929,992] has been shown to prevent the formation of humoral (IgE-like) antibodies in response to an albumin allergic challenge [Martel, R., *Can. J. Physiol. Pharm.* 55:48 (1977)], inhibit murine T-cell activation [Staruch, M., *FASEB* 3:3411 (1989)], prolong survival time of organ gratis in histoincompatible rodents [Morris, R., *Med. Sci. Res.* 17:877 (1989)], and inhibit transplantation rejection in mammals [Calne, R., European Patent Application 401,747]. Rapamycin blocks calcium-dependent, calcium-independent, cytokine-independent and constitutive T and B cell division at the G1-S interface. Rapamycin inhibits gamma-interferon production induced by Il -1 and also inhibits the gamma-interferon induced expression of membrane antigen. [Morris, R. E., *Transplantation Rev.* 6:39 (1992)]. The use of rapamycin in preventing coronary graft atherosclerosis (CGA) in rats has been disclosed by Meiser [*J. Heart Lung Transplant* 9:55 (1990)]. Arterial thickening following transplantation, known as CGA, is a limiting factor in graft survival that is caused by a chronic immunological response to the transplanted blood vessels by the transplant recipient's immune system. [Dec. G, *Transplantation Proc.* 23:2095 (1991) and Dunn, M. *Lancet* 339:1566 (1992)]. The disclosed invention is distinct from the use of rapamycin for preventing CGA, in that CGA does not involve injury to the recipients own blood vessels; it is a rejection type response. The disclosed invention is related to vascular injury to native blood vessels. The resulting intimal smooth muscle cell proliferation dose not involve the immune system, but is growth factor mediated. For example, arterial intimal thickening after balloon catheter injury is believed to be caused by growth factor (PGDF, bFGF, TGFb, IL-1 and others)-induced smooth muscle cell proliferation and migration. [Ip, J. H., *J. Am. Coll. Cardiol* 15:1667 (1990)]. Ferns has also shown that the immune response is not involved in arterial intimal thickening following balloon catheterization, as he found that there was no difference in intimal thickening between arteries from athymic nude rats (rats lacking T-cells) and normal rats after balloon catheterization [*Am. J. Pathol.* 138:1045 (1991)].

DESCRIPTION OF THE INVENTION

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal in need thereof by administering an antiproliferative effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium. Rapamycin is also useful in preventing intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion resulting from mechanically mediated injury. In particular, for the prevention of restenosis following a percutaneous transluminal coronary angioplasty procedure.

Treating includes retarding the progression, arresting the development, as well as palliation. Preventing includes inhibiting the development of and prophylacticly preventing of hyperproliferative vascular disease in a susceptible mammal.

This invention also provides a method of using a combination of rapamycin and mycophenolic acid for the same utilities described above. Mycophenolic acid, an antiproliferative antimetabolite, inhibits inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, enzymes in the de novo purine biosynthetic pathway. This results in an inhibition of DNA synthesis which causes an accumulation of cells at the G 1-S interface. Other combinations containing rapamycin that are useful for preventing or treating hyperproliferative vascular disease will be apparent to one skilled in the art. These include, but are not limited to, using rapamycin in combination with other antiproliferative antimetabolites.

The effect of rapamycin on hyperproliferative vascular disease was established in an in vitro and an in vivo standard pharmacological test procedure that emulates the hyperproliferative effects observed in mammals that are undergoing intimal smooth muscle proliferation and are therefore developing restenosis. Cycloporin A was also evaluated in these test procedures for the purpose of comparison. The combination of rapamycin and mycophenolic acid was evaluated in the in vivo test procedure. The procedures and the results obtained are described below.

Rapamycin and cyclosporin A were evaluated in an in vitro standard pharmacological test procedure which emulates the intimal smooth muscle cell proliferation observed following vascular injury. Results were obtained by measuring DNA and protein synthesis in rat smooth muscle cells that have been stimulated with a growth factor such as fetal calf serum or a hypertrophic mitogen, such as angiotensin II. The following briefly describes the procedure that was used. Rat smooth muscle cells were maintained in a 1:1 mixture of defined Eagle's medium (DEM) and Ham's F12 medium with 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 mg/mL) and 25 mL Hepes at pH 7.4. Cells were incubated at 37° C. in a humidified atmosphere of 5% CO_2 with media changes every 2–3 days. Each compound tested was diluted with an appropriate vehicle to obtain a 1 mM stock solution. Ethanol was used as the vehicle for rapamycin and 20% tween 80 in ethanol was the vehicle for cyclosporin A. Test concentrations of drug were obtained by diluting appropriate concentrations of stock solution with serum free media. The smooth muscle cell culture was maintained in a defined serum free media containing 1:1 DEM and Ham's F12 medium, insulin (5×10^{-7}M), transferrin (5 μg/mL), and ascorbate (0.2 mM) for 72 hours before testing in a multi-well plate. After the 72 hour period, an appropriate quantity of stock solution containing either rapamycin or cyclosporin A was added to the smooth muscle cell culture and media mixture. After a 24 hours the appropriate growth factor was added. For the measurement of DNA synthesis, ^{3}H-thymidine was added at 12 hours after the growth factor was added, and the cells were harvested at 36 hours. For the measurement of protein synthesis, ^{3}H-leucine was added at 14 hours after the growth factor was added, and the cells were harvested at 18 hours. The amount of incorporated radioactive label was measured on a scintillation counter.

The following table shows the results obtained for rapamycin on DNA and protein synthesis in smooth muscle cells that were stimulated with 10% fetal calf serum, as measured by incorporation of tritiated thymidine or leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only was normalized to 100%, and the results for cells treated with fetal calf serum or fetal calf serum plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON DNA AND PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH FETAL CALF SERUM*

	^{3}H-Thymidine Incorporation (% of Media)	^{3}H-Leucine Incorporation (% of Media)
Media	100%	100%
FCS	495%	174%
1000 nM RAP + FCS	136%	95%
100 nM RAP + FCS	172%	91%
10 nM RAP + FCS	204%	74%
1 nM RAP + FCS	403%	106%

*Abbreviations:
RAP = rapamycin;
Media = defined serum free media; and
FCS = 10% fetal calf serum.

The following table shows the results obtained for rapamycin on protein synthesis in smooth muscle cells that were stimulated with 10^{-6} nM angiotensin II, as measured by incorporation of tritiated leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only were normalized to 100%, and the results for cells treated with angiotensin or angiotensin plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH ANGIOTENSIN II*

	^{3}H-Leucine Incorporation (% of Media)
Media	100%
ANG	159%
1000 nM RAP + ANG	53%
100 nM RAP + ANG	57%
10 nM RAP + ANG	61%
1 nM RAP + ANG	60%

*Abbreviations:
RAP = rapamycin;
Media = defined serum free media; and
ANG = 10^{-6} nM angiotensin II.

The results of the standard in vitro test procedure showed that rapamycin had a pronounced antiproliferative effect in the presence of FCS and an anti-hypertrophic effect in the presence of angiotensin II. Following vascular injury, DNA and protein synthesis of smooth muscle cells are necessary for the development of restenosis to occur. These results showed that rapamycin inhibited both DNA and protein synthesis in stimulated smooth muscle cells. An antiproliferative effect was also observed with cyclosporin A; however, at 1000 nM, cyclosporin A was cytotoxic and not merely cytostatic. At 1000 nM, cyclosporin A caused lysis of the smooth muscle cells as evidenced by the presence of lactic acid dehydrogenase in the supernatant of the cell culture. Similar toxicity to smooth muscle cells was not observed for rapamycin.

Rapamycin, rapamycin plus mycophenolic acid, and cyclosporin A were evaluated in an in vivo standard pharmacological test procedure that emulates the vascular injury suffered and restenosis that develops following percutaneous transluminal coronary angioplasty in humans. The ability of a test compound to inhibit restenosis was determined by comparing intimal thickening in mammals treated with test compound following balloon catheterization versus intimal thickening in untreated control mammals after the same test procedure. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest,* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A. W., *Lab, Invest,* 49:208 (1983); Clowes, A. W., J. *Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. The following briefly describes the procedure that was used. The left carotid arteries of male Sprague-Dawley rats were injured with an inflated 2 Fr balloon catheter. During a 14 day postoperative period, these rats were divided into groups and treated daily with rapamycin (1.5 mg/kg; i.p.), rapamycin plus mycophenolic acid (1.5 mg/kg; i.p.+ 40 mg/kg; p.o.), or cyclosporin A (3 mg/kg; i.p.). Treatment was administered on days 0 to 13 postoperatively. Additionally, one group each also received rapamycin (6 mg/kg/day; i.p.) or cyclosporin A (40 mg/kg/day; i.p.) for two days postoperatively, and then received no treatment for the next 12 days. An untreated group was used an injured control to establish the amount of intimal growth in the absence of treatment. The right carotid was used as an uninjured control in all groups. After the 14-day period, the rats were sacrificed, the carotids removed. The mean areas of the intima and blood vessel wall were measured by morphometry. Results are expressed as an intima percent which can be expressed according to the following formula:

$$\frac{\text{area of intima}}{\text{area of vessel}} * 100$$

The following table shows the results that were obtained.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)*

Test Group	Intima Percent ± S.E.
Uninjured Control	0.00 ± 0.00
Untreated Injured Control	33.3 ± 19.66
RAP (1.5 mg/kg - 14 days)	6.78 ± 4.69
RAP (6 mg/kg - 2 days)	16.56 ± 6.22
RAP + MPA (14 days)	1.6 ± 3.5
CsA (3 mg/kg - 14 days)	26.46 ± 27.42
CsA (40 mg/kg - 2 days)	31.14 ± 20.66

*Abbreviations:
RAP = rapamycin;
MPA = mycophenolic acid; and
CsA = cyclosporin A.

These results show that treatment with rapamycin (1.5 mg/kg for 14 days) resulted in an 80% decrease in the mean percentage intimal thickening compared with the untreated injured control group. Similarly, treatment with the combination of rapamycin and mycophenolic acid produced almost a complete inhibition of intimal thickening (95% reduction in intimal thickening compared with untreated injured control). Cyclosporin A failed to produce any meaningful reduction in intimal thickening.

Similar results were obtained when rapamycin was evaluated at different doses in the above in vivo standard phar-

macological test procedure that emulates the vascular injury that occurs following a percutaneous transluminal coronary angioplasty procedure in humans. Rapamycin was administered on postoperative days 0–13, and examination by morphometry was performed on day 14. Rapamycin, at a dose of 1.5 and 3 mg/kg significantly arrested the development of restenosis as measured by the intima percent 14 days after balloon catheterization, whereas restenosis was clearly observed in the untreated injured control group. These results are summarized in the table below.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	6 mg/kg	0–13	30.92 ± 4.06
Rapamycin	3 mg/kg	0–13	22.68 ± 6.28
Rapamycin	1.5 mg/kg	0–13	21.89 ± 4.2

The results of the in vitro and in vivo standard test procedures demonstrate that rapamycin and rapamycin in combination with mycophenolic acid are useful in treating hyperproliferative vascular disease.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium.

Rapamycin and rapamycin plus mycophenolic acid were also evaluated in a modification of the in vivo test procedure described above. In the modified test procedure, treatment with rapamycin or rapamycin plus mycophenolic acid were stopped on day 14, as above, but the animals were not sacrificed immediately. Intimal thickening was observed when the animals were sacrificed 1, 2, 4 weeks, and 44 days alter treatment had been stopped. Microscopic analysis showed that endothelium regeneration had not occurred during the two week treatment period. For example, 44 days after undergoing balloon catheterization procedure of the carotid artery, untreated injured control rats had an intima percent (±S.E.) of 62.85±3.63, and rats treated with rapamycin+mycophenolic acid (1.5/40 mg/kg) on postoperative days 0–13 had an intima percent (±S.E.) of 50.39±2.58. Better results were not obtained when the same regimen was administered on days 0–30 (intima percent (±S.E.) of 53.55± 2.85). Following cessation of treatment with rapamycin or rapamycin plus mycophenolic acid intimal proliferation, that was previously suppressed, was able to occur. These results are consistent with the results shown in the table above, in which treatment for 2 days with rapamycin followed by 12 days of no treatment inhibited intimal thickening to a lesser degree than treatment with rapamycin for 14 days. These results are expected, as in the absence on an integral endothelial layer, the intimal smooth muscle cells will proliferate. It has been shown that intimal smooth muscle cell growth does not have an inhibitory effect on normal endothelial regeneration, and that intimal smooth muscle cell proliferation ceases when the endothelial layer is established. [Reidy, M., *Lab. Invest.* 59:36 (1988); Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979)]. As such, treatment with rapamycin or rapamycin in combination with mycophenolic acid should be employed so long as the beneficial effect is seen. As the degree of restenosis can be monitored by angiographic and sonographic techniques, the dosage necessary to sustain the opened vessels can be adjusted.

To evaluate the ability of rapamycin and rapamycin plus mycophenolic acid to prevent restenosis following an angioplasty procedure, rapamycin was evaluated in the same in vivo standard pharmacological test procedure for restenosis that was described above, except that treatment with rapamycin began three days before (day-3) the angioplasty procedure was performed. The following table shows the results obtained on day 14 following balloon catheterization of the carotid artery on day 0. Results for treatment from day 3 to 13 are also provided.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	1.5 mg/kg	–3–13*	9.85 ± 1.15
Rapamycin	1.5 mg/kg	–3–3	30.7 ± 6.67
Rapamycin	1.5 mg/kg	–3–0	37.31 ± 4.33
Rapamycin	1.5 mg/kg	3–13	44.38 ± 5.49

*Treatment from three days pre-balloon catheterization to day 13 days post-catheterization.

The results in the table above show that rapamycin prevented the development of restenosis following a balloon angioplasty procedure of the carotid artery, when rapamycin was administered from three days pre-angioplasty until day 13. Treatment from day minus 3 until day 3 or day 0 afforded a lesser degree of prevention, and treatment from day 3 to day 13 did not prevent restenosis.

The effect of rapamycin plus mycophenolic acid (MPA) was also evaluated in the angioplasty standard pharmacological test procedure. The table below shows the results obtained where rats underwent a balloon catheterization procedure of the carotid artery on day 0, and were sacrificed and examined morphometrically on day 44. The treatment regimen is described in the table.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 44)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			62.85 ± 3.63
Rapamycin + MPA	40/1.5 mg/kg	0–13	50.39 ± 2.58

-continued

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 44)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Rapamycin + MPA	40/1.5 mg/kg	0–30	53.55 ± 2.85
Rapamycin + MPA	40/1.5 mg/kg	–3–13	18.76 ± 10.6

These results show that treatment with rapamycin and mycophenolic acid from day minus 3 to day 13 did effectively prevent restenosis at day 44, whereas the regimens which did not include drug administration before the angioplasty procedure did not effectively prevent restenosis at day 44.

Similar results were obtained when rat thoracic aortas were subjected to a balloon catheterization procedure, as described above, on day 0. The rats were either sacrificed and examined on day 14 or on day 44. The results obtained with rapamycin and rapamycin plus mycophenolic acid (MPA) are shown in the table below.

EFFECT OF RAPAMYCIN AND RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED THORACIC AORTAS

Group	Dose	Treatment Days	Intima Percent ± S.E.
Day 14 results			
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			15.52 ± 2.99
Rapamycin + MPA	40/1.5 mg/kg	–3–13	0.00 ± 0.00
Day 44 Results			
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			28.76 ± 6.52
Rapamycin	1.5 mg/kg	–3–13	0.00 ± 0.00
Rapamycin + MPA	40/1.5 mg/kg	–3–13	8.76 ± 3.34

The results in the table above show that treatment with rapamycin from 3 days preoperatively until 13 days postoperatively completely prevented the development of restenosis 44 days after a balloon catheterization of the thoracic aorta. Using the same treatment regimen, rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterization and significantly prevented restenosis 44 days following balloon catheterization.

Similarly, day minus 3 to day 13 treatment with rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterizaton of the abdominal aortas in rats. These results are shown in the table below.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED ABDOMINAL AORTAS (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			10.17 ± 2.42
Rapamycin + MPA	40/1.5 mg/kg	–3–13	0.00 ± 0.00

The results in the tables above show that rapamycin, alone or in combination with mycophenolic acid, is useful in preventing restenosis following invasive procedures that disrupt the vascular endothelial lining, such as percutaneous transluminal coronary angioplasty, vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures. These data also show that the administration of rapamycin, alone or in combination with mycophenolic acid, from 3 days pre-catheterization to 13 days post-catheterization, allowed the endothelium to heal, while preventing intimal smooth muscle cell proliferation. That intimal proliferation did not occur 31 days after administration with rapamycin, alone or in combination with mycophenolic acid, had been stopped, demonstrates that the endothelial layer had regenerated, as intimal proliferation stops after the reestablishment of the endothelial layer. The reestablishment of an intact endothelial layer was confirmed by microscopic examination of the previously catheterized arteries after removal at 44 days.

From the data above, it is particularly preferred that treatment begin with rapamycin or rapamycin plus mycophenolic acid before the procedure is performed, and that treatment should continue after the procedure has been performed. The length of treatment necessary to prevent restenosis will vary from patient to patient. For percutaneous transluminal angioplasty procedures, it is preferred that treatment be administered from 3 or more days before the procedure and continuing for 8 or more days after the procedure. It is more preferred that administration will be for 3 or more days before the angioplasty procedure and continuing for 13 or more days after the procedure. The same administration protocol is applicable when rapamycin, alone or in combination with mycophenolic acid, is used to prevent restenosis following vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures.

When rapamycin is employed alone or in combination with mycophenolic acid in the prevention or treatment of hyperproliferative vascular disease, it can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose

solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Rapamycin, alone or in combination with mycophenolic acid, may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. Rapamycin, alone or in combination with mycophenolic acid, may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Rapamycin, alone or in combination with mycophenolic acid can be administered intravascularly or via a vascular stent impregnated with rapamycin, alone or in combination with mycophenolic acid, during balloon catheterization to provide localized effects immediately following injury.

Rapamycin, alone or in combination with mycophenolic acid, may be administered topically as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily intravenous dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.001–25 mg/kg, preferably between 0.005–10 mg/kg, and more preferably between 0.01–5 mg/kg. Projected daily oral dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.005–50 mg/kg, preferably between 0.01–25 mg/kg, and more preferably between 0.05–10 mg/kg. Projected daily intravenous dosages of mycophenolic acid, when used in combination with rapamycin, would be 0.5–75 mg/kg and preferably between 5–50 mg/kg. Projected daily oral dosages of mycophenolic acid, when used in combination with rapamycin, would be 1–75 mg/kg and preferably between 10– 50 mg/kg.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, intravascular, intranasal, intrabronchial, transdermal, or rectal administration will be determined by the administering physician based on experience with the individual subject treated. In general, rapamycin is most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects, and can be administered either as a single unit dose, or if desired, the dosage may be divided into convenient subunits administered at suitable times throughout the day.

What is claimed is:

1. A method of treating restenosis in a mammal resulting from said mammal undergoing a percutaneous transluminal coronary angioplasty procedure which comprises administering an antirestenosis effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

2. A method of preventing restenosis in a mammal resulting from said mammal undergoing a percutaneous transluminal coronary angioplasty procedure which comprises administering an antirestenosis effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

3. The method according to claim **2**, wherein the administration of rapamycin is initiated before the mammal undergoes the percutaneous transluminal coronary angioplasty procedure.

4. The method according to claim **3**, wherein the rapamycin is administered for 3 or more days before the mammal undergoes the percutaneous transluminal coronary angioplasty procedure and said administration continues for 8 or more days following the percutaneous transluminal coronary angioplasty procedure.

5. The method according to claim **4**, wherein the rapamycin is administered for 13 or more days following the percutaneous transluminal coronary angioplasty procedure.

* * * * *

US005545208A

United States Patent [19]

Wolff et al.

[11] **Patent Number: 5,545,208**

[45] **Date of Patent: Aug. 13, 1996**

[54] **INTRALUMENAL DRUG ELUTING PROSTHESIS**

[75] Inventors: **Rodney G. Wolff**, Minnetonka Beach; **Vincent W. Hull**, Ham Lake, both of Minn.

[73] Assignee: **Medtronic, Inc.**, Minneapolis, Minn.

[21] Appl. No.: **171,361**

[22] Filed: **Dec. 21, 1993**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 815,560, Dec. 27, 1991, abandoned, which is a continuation of Ser. No. 486,580, Feb. 28, 1990, abandoned.

[51] **Int. Cl.**[6] **A61F 2/06**; A61M 29/00; A61K 9/22

[52] **U.S. Cl.** **623/1**; 606/195; 623/12; 604/891.1

[58] **Field of Search** 623/1, 11, 12; 606/191–200; 600/36; 604/891.1

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,326,532	4/1982	Hammar .	
4,650,466	3/1987	Luther .	
4,655,771	4/1987	Wallsten .	
4,678,466	7/1987	Rosenwald .	
4,739,762	4/1988	Palmaz .	
4,871,365	10/1989	Dumican .	
4,872,874	10/1989	Taheri .	
4,886,062	12/1989	Wiktor .	
4,892,539	1/1990	Koch .	
4,923,470	5/1990	Dumican .	
4,944,767	7/1990	Barbucci .	
5,015,253	5/1991	MacGregor .	
5,019,090	5/1991	Pinchuk	623/1
5,019,096	5/1991	Fox, Jr. .	
5,028,597	7/1991	Kodama .	
5,047,020	9/1991	Hsu .	
5,053,048	10/1991	Pinchuk .	
5,059,211	10/1991	Stack .	
5,061,275	10/1991	Wallsten .	
5,062,829	11/1991	Pryor .	
5,102,417	4/1992	Palmaz .	
5,152,783	10/1992	Suzuki .	
5,152,784	10/1992	Tsilibary .	
5,165,919	11/1992	Sasaki .	
5,171,262	12/1992	MacGregor .	
5,213,580	5/1993	Slepian .	

FOREIGN PATENT DOCUMENTS

2153235	1/1985	United Kingdom .
8903232	4/1989	WIPO .

Primary Examiner—Debra S. Brittingham
Attorney, Agent, or Firm—Daniel W. Latham; Harold R. Patton

[57] **ABSTRACT**

A prosthesis for insertion into a lumen to limit restenosis of the lumen. The prosthesis carries restenosis-limiting drugs which elute after the device is positioned in the lumen.

10 Claims, 7 Drawing Sheets

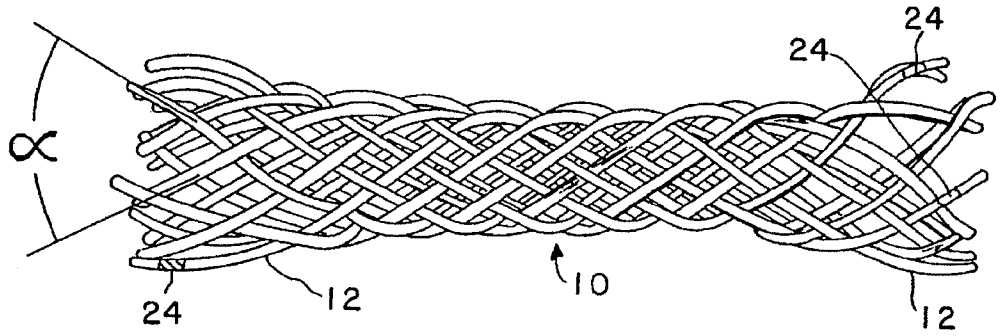


FIG. 1

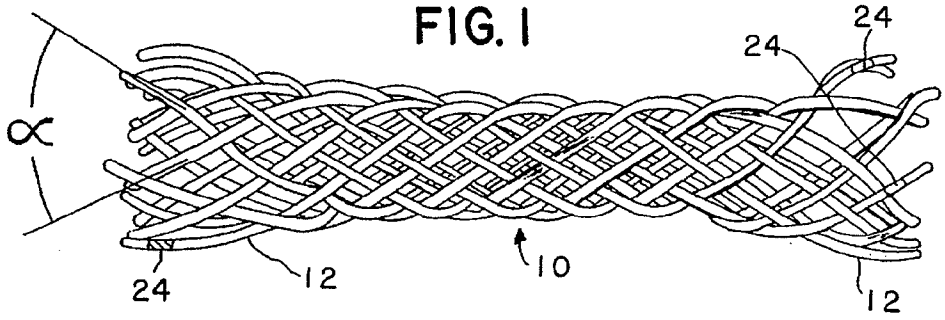


FIG. 2

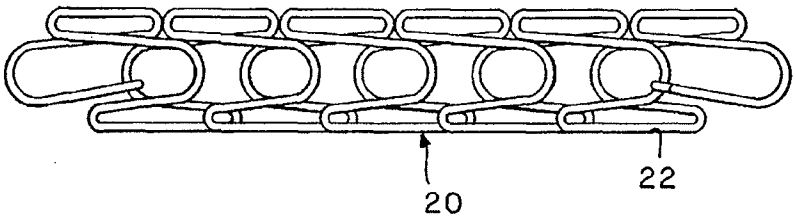


FIG. 3A

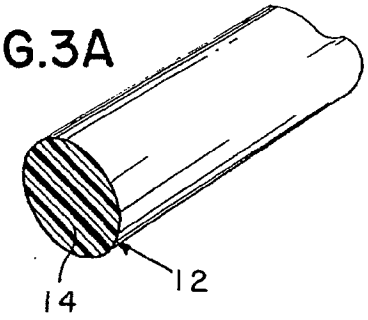


FIG. 4

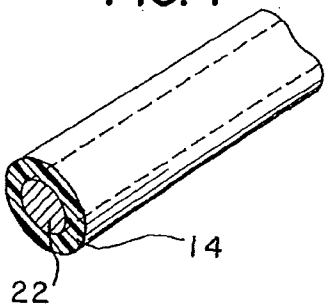


FIG. 5

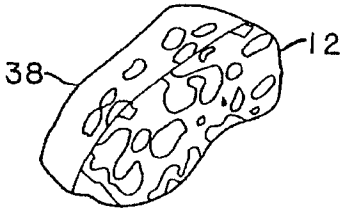


FIG. 3B

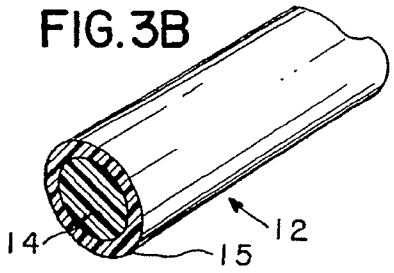


FIG. 6

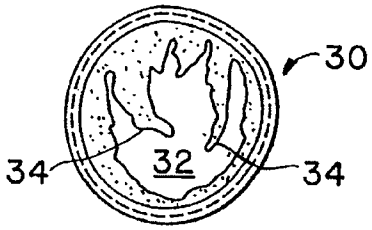


FIG. 7

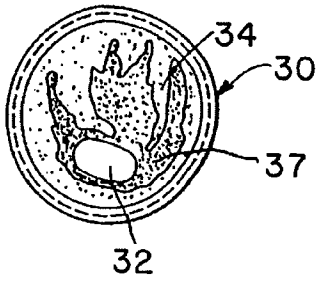


FIG. 8

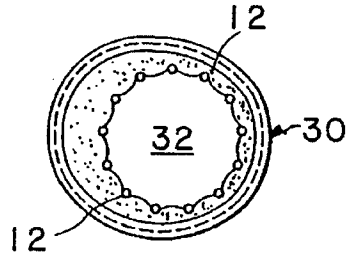


FIG. 9

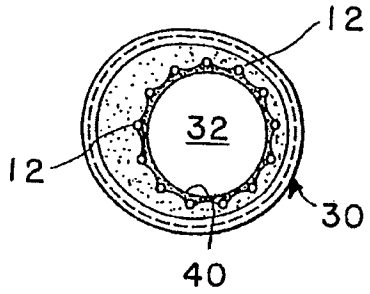


FIG. 10

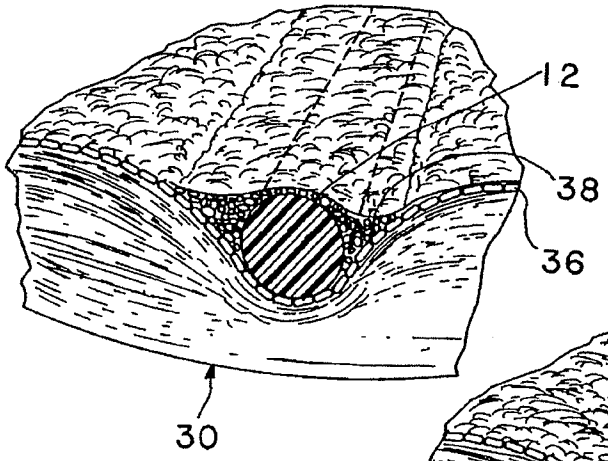


FIG. 11

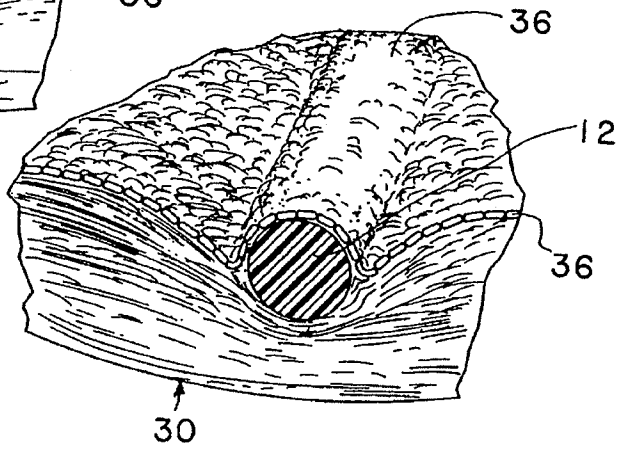


FIG. 12

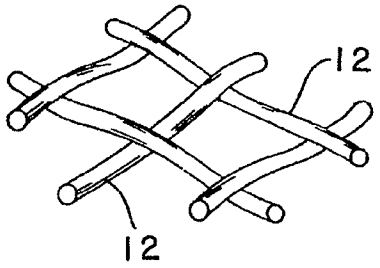


FIG. 13

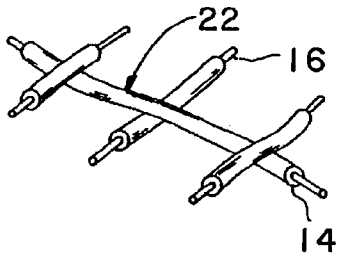


FIG. 14

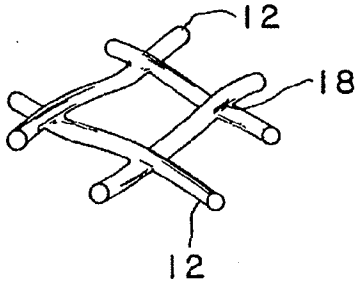


FIG. 15

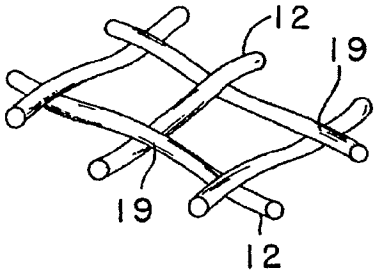


FIG. 16

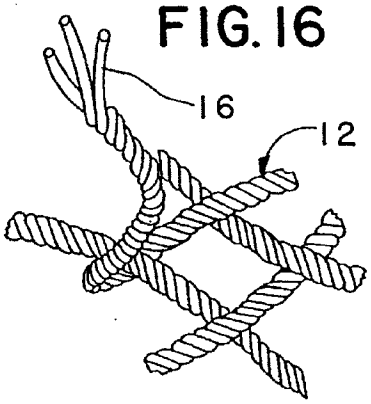
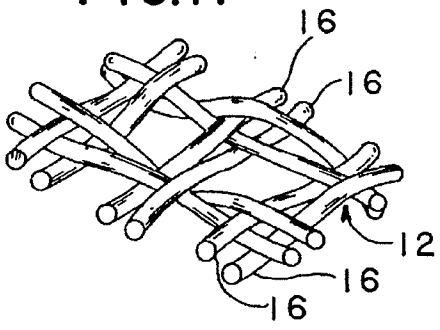


FIG. 17



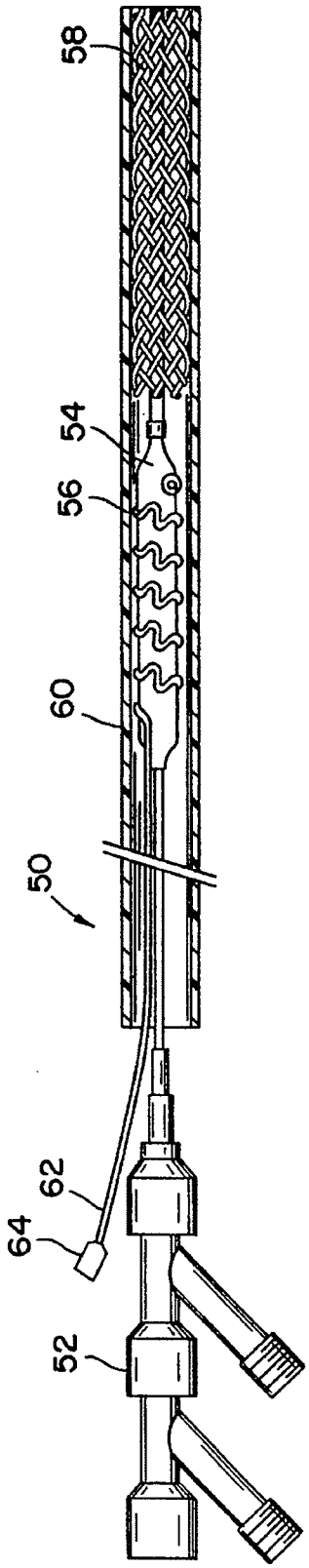


FIG. 18

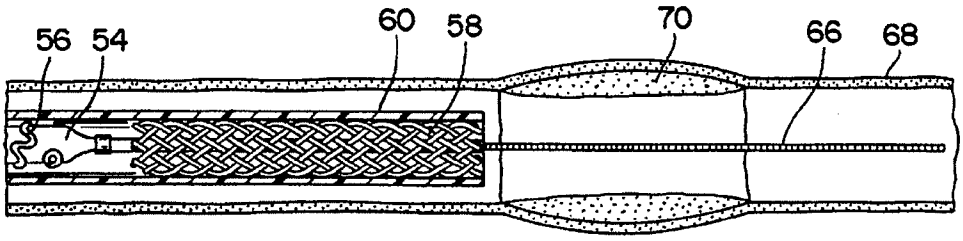


FIG. 19a

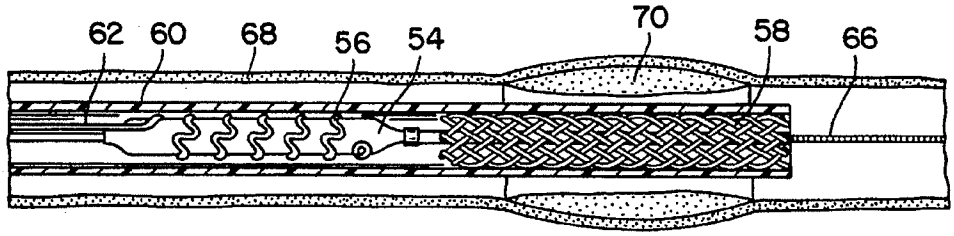


FIG. 19b

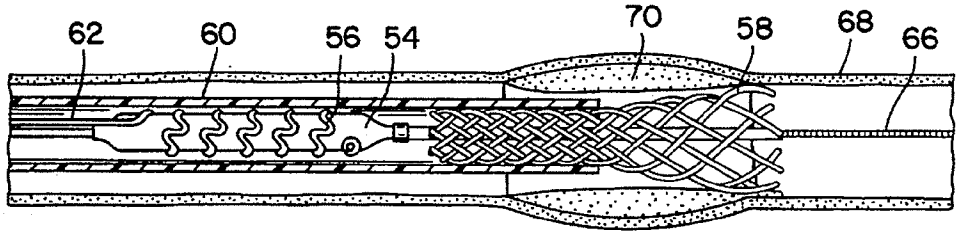


FIG. 19c

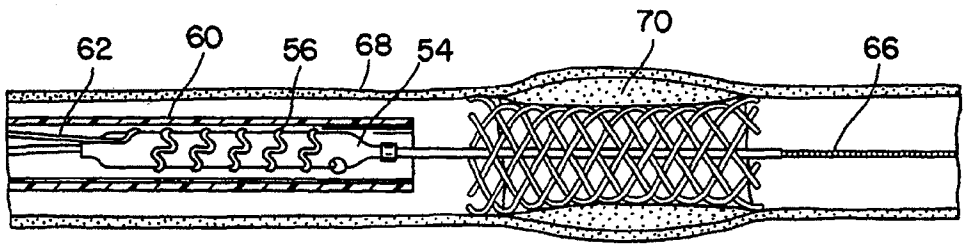


FIG. 19d

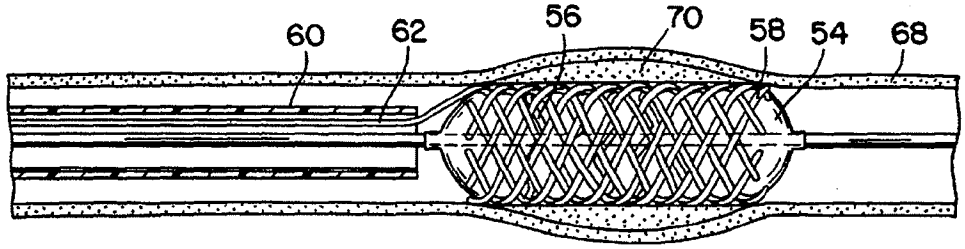


FIG. 19e

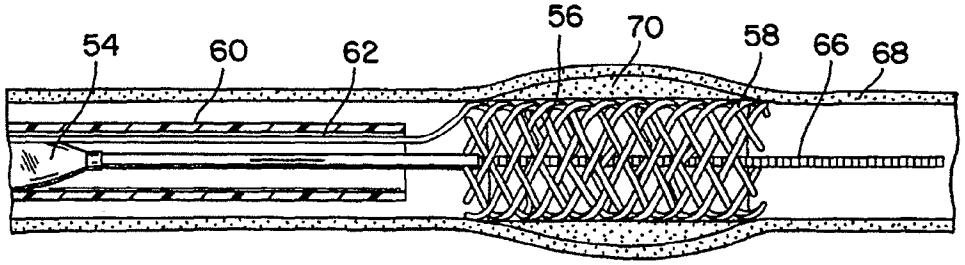


FIG. 19f

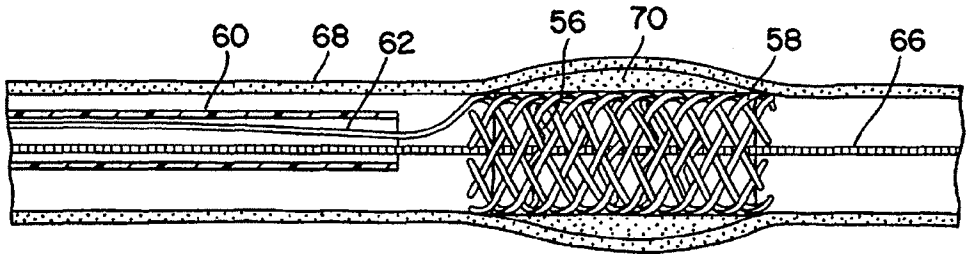


FIG. 19g

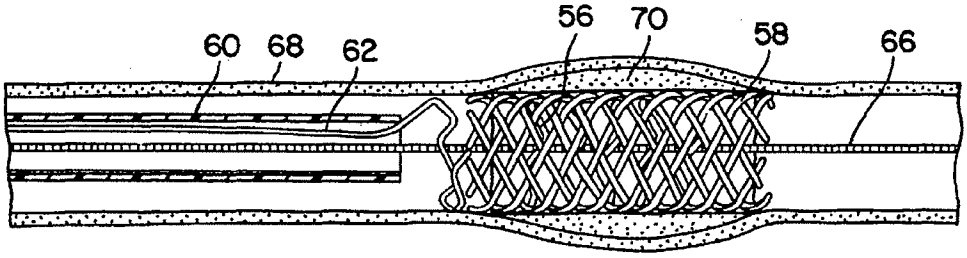


FIG. 19h

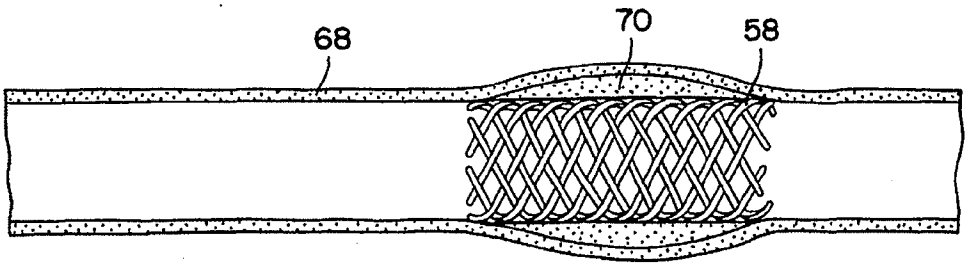


FIG. 19i

INTRALUMENAL DRUG ELUTING PROSTHESIS

This is a continuation-in-part of U.S. Ser. No. 07/815,560, filed Dec. 27, 1991, which is a continuation of U.S. Ser. No. 07/486,580, filed Feb. 28, 1990, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention related to methods for lessening restenosis of body lumens, and to prosthesis for delivering drugs to treat said restenosis.

2. Description of the Related Art

Restenosis is defined as the reclosure of a previously stenosed and subsequently dilated peripheral or coronary vessel. It occurs at a rate of 20–50% for these procedures and is dependent on a number of clinical and morphological variables. Restenosis may begin shortly following an angioplasty procedure, but usually ceases at the end of approximately six (6) months. There is not a current therapeutic procedure that has been proven to significantly reduce this restenosis rate.

A recent technology that has been developed that assesses the problem of restenosis is intravascular stents. Stents are typically metallic devices that are permanently implanted (expanded) in coronary and peripheral vessels. The goal of these stents is to provide a long-term "scaffolding" or support for the diseased (stenosed) vessels. The theory being, if you can support the vessel from the inside, the vessel will not close down or restenose. Unfortunately, initial data from clinical stent implants indicates that these metallic structures are not very successful in reducing restenosis.

Pharmacologic (biochemical) attempts have been made to reduce the rate of restenosis. All of these attempts have dealt with the systemic delivery of drugs via oral, intravascular or intramuscular introduction. Little, if any success has been achieved with this systemic approach.

For drug delivery, it has been recognized for a long period of time that pills and injections may not be the best mode of administration. It is very difficult with these types of administration to obtain constant drug delivery. Patient noncompliance with instructions is also a problem. Through repeated does, these drugs often cycle through concentration peaks and valleys, resulting in time periods of toxicity and ineffectiveness. Thus, localized drug treatment is warranted.

SUMMARY OF THE INVENTION

The invention provides prostheses which may be inserted into a lumen of a body and fixed to the lumen wall adjacent an area needing treatment. Most typically, the lumen will be part of the vascular system which may be subject to restenosis following angioplasty. However, the methods and devices of the invention are also suited to treatment of any body lumen, including the vas deferens, ducts of the gallbladder, prostate gland, trachea, bronchus and liver or any other lumen of the body where medication cannot be applied without a surgical procedure. The invention applies to acute and chronic closure or reclosure of body lumens.

The prostheses of the invention include at least one drug which will release from the device at a controlled rate to supply the drug where needed without the overkill of systemic delivery. The prostheses include means for fixing the device in the lumen where desired. The prostheses may

be completely biodegradable or may be bioabsorbable in whole or incorporated into the lumen wall as a result of tissue over growth, i.e., endothelialization. Alternatively, the prostheses may be biostable in which case the drug is diffused out from the biostable materials in which it is incorporated.

The prosthesis comprises a generally flexible tubular body which is fixed against the lumen walls by a mechanical action. The device should not cause an appreciable reduction in the lumen cross-section where inserted. Conventional stent designs which provide an expansion of the vessel are suitable, though not required. In all cases, the prostheses of the invention require the presence of an elutable drug compounded to the prosthesis itself. With conventional metal stents, the invention requires a drug-carrying coating overlying at least a portion of the metal.

The drugs in the prosthesis may be of any type which would be useful in treating the lumen. In order to prevent restenosis in blood vessels, migration and subsequent proliferation of smooth muscle cells must be checked. Platelet aggregation and adhesion can be controlled with antiplatelets and anticoagulants. Growth factor and receptor blockers and antagonists may be used to limit the normal repair response.

The current invention contemplates the usage of any prosthesis which elutes drugs locally to treat a lumen in need of repair. Controlled release, via a bioabsorbable polymer, offers to maintain the drug level within the desired therapeutic range for the duration of the treatment. When "stent" is referred to herein, it may include the classical definition of stents as they are used in intravascular applications. "Stent" used herein also includes any prothesis which may be inserted and held where desired in a lumen. It includes, but is not limited to, structures such as those shown and described in U.S. Pat. No. 4,886,062 to Wiktor.

BRIEF DESCRIPTION OF THE DRAWINGS

A detailed description of the invention is hereafter described with specific reference being made to the drawings in which:

FIG. **1** is a greatly enlarged side view of an intralumenal drug-eluting prosthesis of the invention;

FIG. **2** is a greatly enlarged side view of an alternative embodiment to the prosthesis of FIG. **1**;

FIG. **3A** is a greatly enlarged fragment of the embodiment of FIG. **1**;

FIG. **3B** is a greatly enlarged fragment of the embodiment of FIG. **1** in which two layers of polymers are present, each having a different drug;

FIG. **4** is a greatly enlarged fragment of the embodiment of FIG. **2**;

FIG. **5** is a greatly enlarged microscopic fragmentary detail of drug shown eluting form the porous structure of a filament or filament coating in a prosthesis into tissue or the vessel lumen;

FIG. **6** is a greatly enlarged cross-section of a blood vessel showing plaque profile immediately post-balloon catheter dilation procedure;

FIG. **7** is a greatly enlarged cross-section of the subject of FIG. **6** at a later date showing restenosis;

FIG. **8** is a greatly enlarged cross-section of a blood vessel showing plaque-prosthesis profile immediately post-prosthesis implant procedure;

FIG. **9** is a greatly enlarged cross-section of the subject of FIG. **8** after ingrowth has occurred;

FIG. **10** is a greatly enlarged fragmentary perspective view of a blood vessel wall and prosthesis filament of FIGS. **1** and **3** immediately after implantation;

FIG. **11** is a greatly enlarged fragmentary perspective view of the subject of FIG. **10** after about one month;

FIG. **12** is a greatly enlarged fragment of a loose weave of prosthesis filaments;

FIG. **13** is a greatly enlarged fragment of a coated metal filament in a loose weave;

FIG. **14** is a greatly enlarged fragment of a melted junction weave of prosthesis filaments in a loose weave;

FIG. **15** is a greatly enlarged fragment of a kinked junction wave of prosthesis filaments;

FIG. **16** is a greatly enlarged fragment of multistrand weave of prosthesis filaments; and

FIG. **17** is a alternative embodiment to FIG. **16**, in which strands are not woven.

FIG. **18** is a partial sectional view of a catheter for delivery of the prosthesis of the present invention.

FIG. **19***a*–**19***i* are sectional views of the deployment of the prosthesis by the catheter of FIG. **18**.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Restenosis

In the discussion above, a very simple definition of restenosis was given. As a complement to this definition, there are several more clinical definitions. Several of these definitions are listed below:

1. Reduction of minimal luminal diameter to less than 50% of the normal lumen diameter.

2. Loss of at least 50% of the initial gain achieved in angioplasty.

3. Decrease of at least 30% in the lumen diameter compared to post-angioplasty result.

4. A return to within 10% of the pre-angioplasty diameter stenosis.

5. An immediate post angioplasty diameter stenosis of less than that increases to 70% or greater at follow-up.

6. Deterioration of 0.72 mm in minimal luminal diameter or greater from post-angioplasty to follow-up.

7. As for 6, but with a deterioration of 0.5 mm.

These definitions are sued by cardiologists to angiographically define restenosis.

Several hypotheses exist on why and how restenosis occurs. The current, most widely accepted explanation is that restenosis is a natural healing process in response to the arterial injury that occurs during all types of angioplasty procedures. This very complex healing process results in intimal hyperplasia, more specifically migration and proliferation of medial smooth muscle cells (SMC). The problem associated with this arterial healing process is that in some instances, it does not shut off. The artery continues to "heal" until it becomes occluded. It should be noted that restenosis is not a re-deposition of the plaque-like cholesterol material that originally occluded the artery.

The following is a possible scenario for restenosis according to the vessel healing hypothesis. Successful angioplasty of stenotic lesions produces cracking of the plaque, dissection into the media, denudation and destruction of endothelial cells, exposure of thrombogenic collagen, released tissue thromboplastin, and an increased loss of prostacyclin production. All of these lead to the aggregation of active platelets.

FIGS. **6** and **7** show a typical vessel **30** in cross-section after angioplasty procedures showing the interior **32** of the lumen. In FIG. **6** the interior of the lumen is rough and includes intimal flaps **34**. Damage causes healing with deposition of platelets, fibrin formation and proliferation of neointima **37** which as shown in FIG. **7** significantly reduces the interior of the lumen.

Activated platelets release several mitogens including platelet derived growth factor (PDGF), epidermal growth factor, and transforming growth factor. PDGF has both mitogenic and chemotactic properties and thus, may induce both mitigation of SMC from the medial layer to the intimal layer as well as proliferation (Intimal hyperplasia). PDGF causes SMC proliferation by binding to specific PDGF receptors. Once the PDGF is bound to the receptors, deoxyribose nucleic acid (DNA) synthesis occurs and new cells are replicated. Minor endothelial injury may cause platelet adhesion and activation with the resultant release of PDGF. Thus, even the deposition of a monolayer of platelets may be sufficient to induce SMC proliferation.

Deeper arterial injury which is sometimes associated with complex stenotic lesions leads to more extensive platelet deposition and activation which may cause an even greater availability of the mitogenic factors. Thus, increased SMC proliferation and intimal hyperplasia. Arterial injury from angioplasty may result in release of PDGF-like compounds from not only platelets but also macrophages, monocytes, endothelial cells, or SMC's themselves.

Activated SMC from human atheroma or following experimental arterial injury secrete PDGF-like molecules which appears to lead to self perpetuation of SMC proliferation by the release of their own PDGF-like substances. Thus, any or all of the cells which can secrete PDGF relate substances (platelets, macrophages, monocytes, endothelia, and smooth muscle cells) may contribute to the cascading effect of restenosis after angioplasty.

The previous restenosis scenario resulted from normal angioplasty procedures. During balloon angioplasty if the balloon is undersized or not totally inflated and the plaque cracking and extensive endothelial denudation does not occur the lesion could still restenose. Rheologic factors contribute as well to the interaction between platelets and the arterial wall. Residual stenosis, resulting from inadequate balloon expansion, produces a high local shear rate and enhances platelet deposition and activation. These stenoses may be important as a stimulus for some proliferation through enhanced platelet deposition and secretion of growth factors. This hypothesis correlates with the increased incidence of restenosis in patients with high-grade residual stenoses or transtenotic gradients.

Prevention of Restenosis

In order to prevent restenosis, one must stop the proliferation of smooth muscle cells. As stated earlier, this is a biochemical process which cannot be treated mechanically. Several hypothesis exist on how to biochemically stop restenosis. Some of which are:

1. Reduce the adhesion and aggregation of the platelets at the arterial injury site.

2. Block the expression of the growth factors and their receptors.

3. Develop competitive antagonists of the above growth factors.

4. Interfere with the receptor signaling in the responsive cell.

5. Find a "natural" inhibitor of smooth muscle proliferation.

Item #1 is directly related to the formation of thrombus, a major problem with all types of angioplasty procedures. Items #2, #3 and #4 are closely related. They deal with blocking restenosis during the massive cell migration and replication cycle. Unlike item #1, these items address the growth factors that are produced from sources other than platelets. Item #5 is listed to address the question; Why don't 50–80% of the people undergoing angioplasty restenose? There may be some type of natural inhibitor that these people produce that stops the proliferation of smooth muscle cells.

There are at least two (2) different ways to prevent the adhesion and aggregation of platelets. One method is to use an antiplatelet and another is to use an anticoagulant.

Antiplatelet drugs include such as aspirin and dipyridamole. Aspirin is classified as an analgesic, antipyretic, anti-inflammatory, antiplatelet drug. It has been clinically tested and proven to reduce the risk of sudden death and/or non-fatal reinfarction in post myocardial infarction (heart attack) patients. The proposed mechanism of how aspirin works, relates directly to the platelets. It somehow blocks the platelets, restricting coagulation. This prevents the cascading platelet aggregation found in thrombus and subsequently restenosis. Aspirin is therefore a possible restenosis inhibitor. Dipyridamole is a drug similar to aspirin, in that is has anti-platelet characteristics. Dipyridamole is also classified as a coronary vasodilator. It increases coronary blood flow by primary selective dilatation of the coronary arteries without altering systemic blood pressure or blood flow in peripheral arteries. These vasodilation characteristics are thought to be possibly beneficial for restenosis prevention.

Anticoagulant drugs include Heparin, Coumadin, Protamine, and Hirudin. Heparin is the most common anticoagulant used today. Heparin, in one form or another, is used in virtually every angioplasty procedure performed. All four (4) of these drugs function as an anticoagulant by preventing the production of thrombin, a binding agent which causes blood to clot. This too, may reduce the cascading effect of platelet aggregation at the lesion site, thus possibly reducing restenosis. The use of Protamine in the presence of Heparin causes the Protamine to function as a Heparin antagonist, blocking the effect of the Heparin. Protamine, however, used alone, acts as an anticoagulant. Hirudin is singled out because it is not normally found in the human body. Hirudin is a drug that is found in the salivary glands of leeches. It is a very concentrated anticoagulant that functions in a similar manner as Heparin, Coumadin, and Protamine.

There are several types of drugs that interrupt cell replication. Antimitotics (cytotoxic agents) work directly to prevent cell mitosis (replication), whereas antimetabolites prevent deoxyribose nucleic acid (DNA) synthesis, thus preventing replication. The action of the antimitotics and antimetabolites are similar, they can be grouped into one category. This category will be known as the anti-replicate drugs.

Anti-replicate drugs include among others: Methotrexate, Colchicine, Azathioprine, Vincristine, VinBlastine, Fluorouracil, Adriamycin, and Mutamycin. The target systemic molarity desired with methotrexate is on the order of 10^{-6}M with a range of between 10^{-3} to 10^{-8} Molar. Locally, the molarity of the drug may be highly variable, which is one of the great disadvantages in systemic administration of the drug. When drugs are delivered locally via the prosthesis of the invention, they may be at therapeutic levels at the diseased site while at the lower limits of detectability in the bloodstream. So little drug is required for effective local treatment of a lumen that the drug may not be detectable in blood samples.

Anti-inflammatory drugs such as glucocorticoids (e.g., dexamethasone, betamethasone) can also be useful to locally suppress inflammation caused by injury to luminal tissue during angioplasty.

If the restenosis process ranges from shortly after injury to about four to six months later, then the generalized elution rates contemplated are such that the drug should ideally start to be released immediately after the prosthesis is secured to the lumen wall to lessen cell proliferation. The drug should then continue to elute for up to about four to six months in total.

Complex systems of drugs may be carried by the prosthesis. An anticoagulant or antiplatelet may be included in the outermost surface of the device in order to elute off very quickly for the first several days. Antiinflammatories and antireplicates can be formulated into the device to continue to elute later, when in contact with non-blood cells after neointima overgrowth has surrounded the device. This usually occurs in about two weeks. The drug elution rate does not need to be uniform, and may be tailored to fit the need of the patient.

Prosthesis (Stent) Design

The current invention contemplates the usage of any prosthesis which elutes drugs locally to treat a lumen in need of repair. When "stent" is referred to herein, it may include the classical definition of stents as they are used in intravascular applications. "Stent" used herein also includes any prosthesis which may be inserted and held where desired in a lumen.

FIGS. **1** through **17** show features of some of the prostheses which may be used to carry and elute restenosis limiting-drugs.

The current preferred stent **10** configuration consists of a single filar, monofilament braided mesh design as shown in FIG. **1**. There are sixteen (16) filaments **12**, eight (8) of which are wound in one helical direction, and the remaining eight (8) which are wound in the opposite direction. The stent **10** is self-expanding to a predetermined diameter. The profile (diameter) of the stent **10** can be easily reduced by pulling the stent **10** longitudinally. In this reduced profile configuration, the stent **10** can be loaded into a catheter for delivery into the vessel.

The stent **20** shown in FIGS. **2** and **4** is a metallic malleable design which may be forced against a lumen wall by a balloon catheter which fixes it into position. The exterior surface of the metal filaments **22** would include a coating **14** with a drug-eluting polymer described previously. The polymer may be biostable or bioabsorbable. If biostable, the drug would diffuse out of the polymer.

The variations of design shown in the embodiments of FIGS. **1** and **2** show that the prosthesis of the invention must be secured against a lumen wall and must carry a drug-eluting polymer.

There are many variables in the design of stent **10**. The angle (a) of the filaments **12** is a major variable. The angle a can vary from 0 degrees to 180 degrees. The design in the Figures is based on an angle in the 60 degree to 90 degree range.

There are many options for fabricating the drug eluting stents. One option is to have all sixteen (16) filaments be drug eluting. Or, you could have any number of filaments up to sixteen (16) degrade and elute drugs. Another option is to have a multi-filar stent. Instead of a single filament braided into the stent, it is possible to have two (2), three (3), or even four (4) strands 16 braided to form a filament **12** as shown in FIG. **16**. This would create a stent with much greater expansile force, but also have much more material in the surface area. This is a common trade-off in stent design. Similar to the single-filar design, the multi-filar form shown in FIG. **16** could have varying numbers of strands **16** that are drug eluting. FIGS. **16** and **17** show that the multi-filar design may be braided or unbraided. One (1), two (2), three (3), or four (4) of the filaments could be impregnated with a drug and biodegradably elute. Alternatively, the polymer may be biostable which allows for diffusion of the drug without degradation.

The stent **10** of FIG. **1** consists of a wound braided mesh which is self-expanding to a predetermined diameter and whose profile diameter can be greatly reduced for catheter introduction. The radial expansile force increases with diameter to the point of the self-expanded diameter limit, at which point the angle between the filaments and the longitudinal axis is a maximum. FIGS. **12** and **15** show alternative construction techniques to alter the radial expansive force. FIG. **12** shows the filaments **12** being woven without any connection. FIG. **13** is similar except the filament **22** is formed with a metal core **16** and a-coating **14**. In FIG. **14** the individual filaments **12** are shown with a bonded juncture **18**. The bonding at the juncture **18** prevents the individual filaments **12** from sliding relative to each other, which improves the radial strength. The mechanically kinked junction **19** shown in FIG. **15** also limits the sliding of the filaments to change the radial strength. A heated platen press may be pressed against the wound stent while still on the forming mandrel to form the kinks. Higher temperatures may be used to form the melted junctures **18**.

The devices may be made more visible under fluoroscopy and x-ray by incorporating radiopaque materials into marker band **24** to the individual filaments **12** at the ends of the stent **10** as shown in FIG. **1**. Such marker bands could help to locate the stent and assure proper placement and patency.

Bioabsorbable Prosthesis (Stent) Materials

Controlled release, via a bioabsorbable polymer, offers to maintain the drug level within the desired therapeutic range for the duration of the treatment. In the case of stents, the prosthesis materials will maintain vessel support for at least two weeks or until incorporated into the vessel wall even with bioabsorbable, biodegradable polymer constructions.

Several polymeric compounds that are known to be bioabsorbable and hypothetically have the ability to be drug impregnated may be useful in prosthesis formation herein. These compounds include: poly-1-lactic acid/polyglycolic acid, polyanhydride, and polyphosphate ester. A brief description of each is given below.

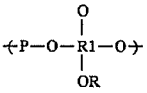
Poly-1-lactic acid/polyglycolic acid has been used for many years in the area of bioabsorbable sutures. It is currently available in many forms, i.e., crystals, fibers, blocks, plates, etc. These compounds degrade into non-toxic lactic and glycolic acids. There are, however, several problems with this compound. The degradation artifacts (lactic acid and glycolic acid) are slightly acidic. The acidity causes minor inflammation in the tissues as the polymer degrades. This same inflammation could be very detrimental in coronary and peripheral arteries, i.e., vessel occlusion. Another problem associated with this polymer is the ability to control and predict the degradation behavior. It is not possible for the biochemist to safely predict degradation time. This could be very detrimental for a drug delivery device.

Another compound which could be used are the polyanhydrides. They are currently being used with several chemotherapy drugs for the treatment of cancerous tumors. These drugs are compounded into the polymer which is molded into a cube-like structure and surgically implanted at the tumor site.

Polyanhydrides have weaknesses in their mechanical properties, due to low molecular weights. This drawback makes them difficult to process into a filament form. Also, polyanhydrides have poor solubility, making characterization and fabrication difficult.

The compound which is preferred is a polyphosphate ester. Polyphosphate ester is a compound such as that disclosed in U.S. Pat. Nos. 5,176,907; 5,194,581; and 5,656,765 issued to Leong which are incorporated herein by reference. Similar to the polyanhydrides, polyphoshate ester is being researched for the sole purpose of drug delivery. Unlike the polyanhydrides, the polyphosphate esters have high molecular weights (600,000 average), yielding attractive mechanical properties. This high molecular weight leads to transparency, and film and fiber properties. It has also been observed that the phosphorous-carbon-oxygen plasticizing effect, which lowers the glass transition temperature, makes the polymer desirable for fabrication.

The basic structure of polyphosphate ester monomer is shown below.



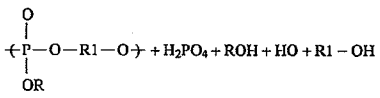
where

P corresponds to Phosphorous,

O corresponds to Oxygen,

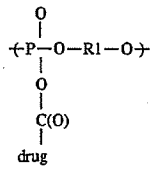
and R and R1 are functional groups.

Reaction with water leads to the breakdown of this compound into monomeric phosphates (phosphoric acid) and diols (see below).

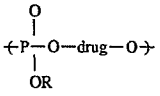


It is the hydrolytic instability of the phosphorous ester bond which makes this polymer attractive for controlled drug release applications. A wide range of controllable degradation rates can be obtained by adjusting the hydrophobicities of the backbones of the polymers and yet assure biodegradability.

The functional side groups allow for the chemical linkage of drug molecules to the polymer. This is shown below.



The drug may also be incorporated into the backbone of the polymer.



In summary, the highly hydrolytically reactive phosphorous ester bond, the favorable physical properties, and the versatile chemical structure make the polyphosphate esters a superior drug delivery system for a prosthesis.

FIGS. **3A** and **3B** show that the filaments **12** may be made from one or several layers of polymer. In FIG. **3A** only a single polymer is present to carry the drugs. In FIG. **3B** a second layer of polymer **15** is shown. That layer **15** may be a simple barrier which limits diffusion of drugs in the polymer **14**. In that event, the smaller molecules could elute out immediately, while larger compounds would not elute until later when the layer **15** has biodegraded. Alternatively, layer **15** may include a different drug incorporated therein from that found in layer **14**. The barrier coating **15** could be as simple as a silicone or polyurethane.

Operation

The prosthesis is inserted into the lumen wherever needed as per the usual procedure for stents. The device is fixed into place either by radial expansion in devices such as shown in FIG. **1** or are deformed by a balloon catheter in the case of devices in accordance with FIG. **2**.

FIGS. **8** through **11** show the placement and effects of the drug-eluting prosthesis of the invention. The prosthesis tacks up any intimal flaps and tears caused by any prior ballooning. The initial deposition of platelets and subsequent thrombus formation **38** is controlled and minimized by the stent design and the elution which limits platelet aggregation and other immediate repair responses described previously. Localized thrombus formations in the areas of cracked and roughened plaques and newly exposed underlying collagen and fibro-muscular tissues is also decreased. This results in limited but quick neointima formation **40** and intimal proliferation over individual stent filaments progressing to mature endothelial lining. Long term significant restenosis is therefore limited. Elution of the anti-replicates along or in conjunction with the initial elution of anticoagulants can also limit the extent of the restenosis which occurs in the natural healing process.

In yet another embodiment of the invention, a purely polymeric prosthesis such as that having the configuration shown in FIG. **1** can be combined with an expandable metal stent to provide additional support for the prosthesis. This can be important since preferred bioabsorbable polymeric materials for the prosthesis may have insufficient resilience to expand an occluded body lumen or to maintain its expansion. By including a metal stent within the lumen of the polymeric prosthesis, the polymeric prosthesis is effectively held against the wall of the body lumen by the strength of the metal stent. In a most preferred aspect of this embodiment, the metal stent is only temporarily implanted so that only the bioabsorbable prosthesis remains implanted in the body lumen on a long term basis. This can be accomplished by including a polymeric stent body and a metal stent body on the distal end of a catheter designed to expand and release the stents. Both of the stent bodies have a number of support elements which make up an open-ended, radially expandable self-supporting tubular structure. In the polymeric stent structure, a bioabsorbable polymeric element (such as a filment made from a bioabsorbable polymer) having drugs incorporated therein can be attached to the support elements of the body so that at least a portion of the bioabsorbable element is exposed at the exterior surface of the polymeric stent body. The stents are arranged on the distal end of the catheter such that the catheter can provide remote, transluminal deployment of the stents, with the metal stent inside the polymeric stent, from an entry point into a selected portion of the body lumen to be treated and also remote actuation of an expansion mechanism from the proximal end of the catheter. The expansion mechanism (e.g. a balloon or the like if the metal stent is made of malleable metal for balloon expansion or a release mechanism if the metal stent is a self-expanding stent made from a resilient metal) is one capable of providing radial expansion of the metal stent body to bring the metal stent into supporting contact with the polymeric stent body and also to press the polymeric stent body so that it expands radially into contact with the wall of the body lumen. This will bring the bioabsorbable element into supporting contact with a body lumen at an interior portion of the body lumen to be treated and will position the bioabsorbable element to deliver drugs to the body lumen. Following the expansion of the stents into luminal contact, the balloon (if the expansion device is a balloon) can be deflated which allows luminal flow to be restored. After the stents have been in place for a predetermined period of time, the metal stent can be removed to leave only the polymeric stent (and its drug delivery component) in position in the body lumen. This can be accomplished, for example, by radially contracting the metal stent and then withdrawing it from the body lumen or by unwinding the metal stent a bit at a time as it is withdrawn from the body lumen.

Referring now to specific embodiments shown in the drawings, one possible configuration for a polymeric prosthesis supported by a metal stent is that of the prosthesis shown in FIG. **1** supported by a metal stent having a configuration such as that taught in U.S. Pat. No. 4,886,062 to Wiktor which is incorporated herein by reference. These devices may be combined by simply placing the polymeric prosthesis over the metal stent and balloon; introducing the prosthesis, stent, and balloon into the body lumen as a unit until it reaches the desired point for expansion; inflating the balloon to radially expand the prosthesis and stent into contact with the body lumen; and removing the balloon. In such an embodiment, the metal stent would be permanently implanted with the polymeric prosthesis. Another embodiment of this concept is shown in FIGS. **18** and **19*a*–19*i*** in which the metal stent is only implanted for a limited period of time and then removed. Referring now to FIGS. **18** and **19*a*–19*i***, a catheter assembly **50** includes a hub assembly **52** at a proximal end, an inflatable balloon **54**, a metal stent **56** and a polymeric prosthesis **58** at a distal end and a sheath **60** extending from the proximal to the distal end. The metal stent **56** is crimped onto the balloon **54** and includes an

elongated lead **62** extending to the proximal end of the catheter assembly **50** where it includes an enlarged portion **64** to enable an operator to securely grip the lead **62**. Distal to the balloon **54** is the polymeric prosthesis **58** which is constrained from radial self-expansion by the sheath **60**. In operation, a guidewire **66** is inserted into the body lumen **68** and through the point of occlusion **70**. The catheter assembly **50** is then passed into the lumen **68** on the guidewire **66** until the prosthesis **58** is positioned at the point of the occlusion **70**. The sheath **60** is then drawn back, thereby allowing the prosthesis **58** to be pushed out of the sheath **60** by the leading edge of the balloon assembly where it radially self-expands into luminal contact. The balloon **54** and stent **56** are then advanced out of the sheath **60** as a unit and into the open center of the prosthesis **58**. The balloon **54** is then inflated to expand the metal stent **56**, the prosthesis **58** and the occlusion **70**. The balloon **54** is then deflated and withdrawn from the metal stent **56** and prosthesis **58**, leaving the metal stent **56** inside the prosthesis **58** in support of the prosthesis **58** and the occlusion **70**. If the body lumen **68** is a blood vessel, blood flow is restored by deflating the balloon **54**. If desired, the balloon **54** can then be withdrawn entirely from the sheath **60** and also, if desired, the sheath **60** and guidewire **66** can be withdrawn. However, it is preferred to leave the balloon **54**, sheath **60** and guidewire **66** in place in order to provide support for the lead **62** and to avoid entangling the lead **62** with the catheter lumen or guidewire **66** as they are withdrawn. If the balloon **54** or guidewire **66** are to be withdrawn, it may be preferable to modify the sheath **60** by providing a separate lumen in the sheath **60** or another location in the catheter assembly **50** for the lead **62**. After a desired period of time which allows the prosthesis to achieve a stable support for the lumen, the lead **62** is pulled at the proximal end of the catheter assembly **50**, thereby causing the metal stent **56** to unwind and be taken up into the sheath **60**. The metal stent chosen for use in this method should include no edges or ends which can snag the prosthesis **58** and pull it from its intended position in the body lumen **68**. The Sheath is then withdrawn, leaving the prosthesis **58** in place in the lumen **68**.

This completes the description of the preferred and alternate embodiments of the invention. Those skilled in the art may recognize other equivalents to the specific embodiments described herein which equivalents are intended to be encompassed by the claims attached hereto.

We claim:

1. A device for local intralumenal administration of drugs comprising:

(a) a catheter having proximal and distal ends;

(b) a body including a plurality of support elements forming a open-ended, radially expandable, self-supporting tubular structure having an interior surface and an exterior surface;

(c) at least one flexible, polymeric filament attached to the support elements of the body, at least a portion of the filament exposed at the exterior surface of the tubular body, said body mounted on the catheter at the distal end thereof;

(d) means at the proximal end of the catheter to provide sufficient radial expansion of the tubular body to bring the tubular body and polymeric filament into contact with a body lumen at an interior portion of the body lumen to be treated and for releasing the tubular body from the catheter; and

(e) a drug compounded into the polymeric filament such that the drug is delivered to the body lumen when the tubular body is radially expanded into contact with the portion of the body lumen to be treated.

2. The device of claim **1** wherein the support elements of the tubular body are arranged in a helically wound structure.

3. The device of claim **2** wherein the helically wound structure includes a plurality of helical elements, each of which is wound in a helix configuration along a center line of the tubular body as a common axis, said elements wound in opposing helical directions such that the tubular body is variable in radial diameter under axial movement of opposite ends of the body relative to each other.

4. The device of claim **3** wherein the helical elements are made from a bioabsorbable polymer.

5. The device of claim **2** wherein the support elements are made from a deformable metal.

6. The device of claim **1** wherein the polymeric filament is made from a bioabsorbable polymer.

7. The device of claim **1** also comprising a second polymeric filament having the drug compounded therein such that the drug is delivered to the body lumen more rapidly from the second polymeric filament than from the polymeric filament.

8. The device of claim **1** also comprising a barrier coating of polymeric material on the drug-containing filament to limit the rate of drug elution.

9. The device of claim **1** wherein the drug is selected from the group consisting of antiplatelet drugs, anticoagulant drugs, anti-inflammatory drugs, antireplicate drugs and combinations of said drugs.

10. The device of claim **9** wherein the polymeric filament includes a drug selected from the group consisting of anticoagulant drugs and antiplatelet drugs and wherein the device also comprises a second polymeric filament having a drug compounded therein selected from the group consisting of antiinflammatory drugs and antireplicate drugs such that the drug in the polymeric filament is delivered to the body lumen more rapidly than from the second polymeric filament.

* * * * *

US005563145A

United States Patent [19]

Failli et al.

[11] **Patent Number: 5,563,145**

[45] **Date of Patent: Oct. 8, 1996**

[54] **RAPAMYCIN 42-OXIMES AND HYDROXYLAMINES**

[75] Inventors: **Amedeo A. Failli**, Princeton Junction, N.J.; **Guy A. Schichser**, Yardley; **Oleg I. Bleyman**, Holland, both of Pa.

[73] Assignee: **American Home Products Corporation**, Madison, N.J.

[21] Appl. No.: **350,557**

[22] Filed: **Dec. 7, 1994**

[51] **Int. Cl.**[6] **A61K 31/445**; C07D 491/16

[52] **U.S. Cl.** **514/291**; 540/456; 546/14; 514/183

[58] **Field of Search** 540/456; 514/183, 514/291

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,929,992	12/1975	Sehgal et al.	424/122
3,993,749	11/1976	Sehgal et al.	424/122
4,316,885	2/1982	Rakhit	424/122
4,375,464	3/1983	Sehgal et al.	424/122
4,401,653	8/1983	Eng	424/114
4,650,803	3/1987	Stella et al.	514/291
4,885,171	12/1989	Surendra et al.	424/122
5,023,262	6/1991	Caufield et al.	514/291
5,023,263	6/1991	Von Burg	514/291
5,023,264	6/1991	Caufield et al.	514/291
5,078,999	1/1992	Warner et al.	424/122
5,080,899	1/1992	Sturm et al.	424/122
5,091,389	2/1992	Ondeyka et al.	514/291
5,100,883	3/1992	Schiehser	514/183
5,100,899	3/1992	Calne	514/291
5,102,876	4/1992	Caufield	514/183
5,118,677	6/1992	Caufield	514/183
5,118,678	6/1992	Kao et al.	514/183
5,120,842	6/1992	Faillie et al.	540/452
5,130,307	7/1992	Failli et al.	514/321
5,138,051	8/1992	Hughes et al.	540/456
5,151,413	9/1992	Caufield et al.	514/63
5,169,851	12/1992	Hughes et al.	514/291
5,177,203	1/1993	Failli et al.	540/456
5,194,447	3/1993	Kao	514/542
5,221,670	7/1993	Caufield	514/183
5,233,036	8/1993	Hughes	540/455
5,260,300	11/1993	Hu	514/291
5,302,584	4/1994	Kao et al.	514/80
5,373,014	12/1994	Failli et al.	514/291
5,378,836	1/1995	Kao et al.	540/456
5,385,908	1/1995	Nelson et al.	514/291
5,385,909	1/1995	Nelson et al.	514/291
5,385,910	1/1995	Ocain et al.	514/291

FOREIGN PATENT DOCUMENTS

507555A1	7/1992	European Pat. Off.	540/456
WO9405300	3/1994	WIPO	540/456

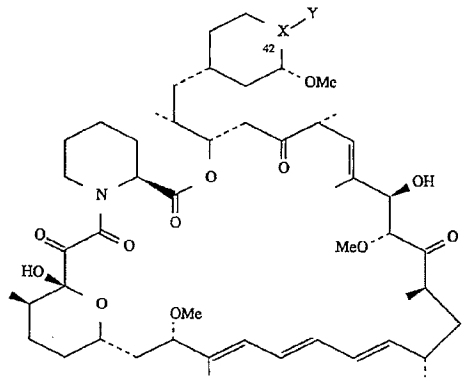
OTHER PUBLICATIONS

Vezina, C., J. Antibiot. 28:721 (1975).
Sehgal, S. N., J. Antibot. 28:727 (1975).
Baker, H. J., Antibiot. 31:539 (1978).
Martel, R. R., Can. J. Physiol. Pharmacol. 55:48 (1977).
Staruch, M. J., FASEB 3:3411 (1989).
Calne, R. Y., Lancet 1183 (1978).
Morris, R. E., Med. Sci. Res. 17:877 (1989).
Baeder, W. L., Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract) (1990).
Meiser, B. M., J. Heart Lung Transplant. 11 (pt. 2):197 (1992).
Stepkowski, S. M., Transplantation Proc. 23:507 (1991).

Primary Examiner—Alan L. Rotman
Attorney, Agent, or Firm—Arnold S. Milowsky

[57] **ABSTRACT**

A compound of the structure



wherein X-Y is C=NOR[1] or CHNHOR[2];

R[1] and R[2] are each, independently, hydrogen, alkyl, alkenyl, alkynyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, cycloalkyl, alkyloxy, alkoxyalkyl, cycloalkylaminoalkyl, cyanoalkyl, fluoroalkyl, trifluoromethylalkyl, trifluoromethyl, ArO—, —$(CH_2)_m$Ar, or —COR[3];

R[3] is alkyl of 1–6 carbon atoms, —NH_2, —NHR^4, —NR^4R^5, or –OR^4;

R[4] and R[5] are each, independently, alkyl, Ar or if both are present can be taken together to form a 4–7 membered ring;

Ar is an aryl or heteroaryl radical which may be optionally substituted; and m=0–6; or a pharmaceutically acceptable salt thereof, which is useful as an immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agent.

15 Claims, No Drawings

RAPAMYCIN 42-OXIMES AND HYDROXYLAMINES

BACKGROUND OF THE INVENTION

This invention relates to rapamycin 42-oximes and hydroxylamines and a method for using them for inducing immunosuppression, and in the treatment of transplantation rejection, graft vs. host disease, autoimmune diseases, diseases of inflammation, adult T-cell leukemia/lymphoma, solid tumors, fungal infections, and hyperproliferative vascular disorders.

Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31,539 (1978); U.S. Pat. No. 3,929,992; and U.S. Pat. No. 3,993,749].

Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

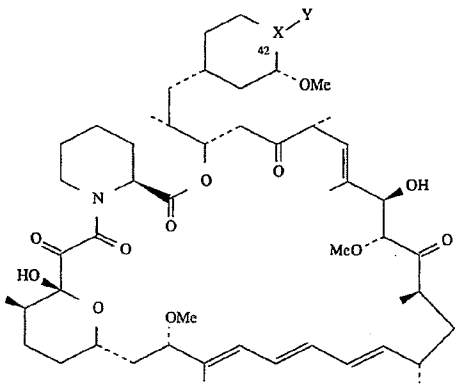
The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Pat. No. 5,100,899].

Rapamycin has also been shown to be useful in preventing or treating systemic lupus erythematosus [U.S. Pat. No. 5,078,999], pulmonary inflammation [U.S. Pat. No. 5,080,899], insulin dependent diabetes mellitus [Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract), (1990)], smooth muscle cell proliferation and intimal thickening following vascular injury [Morris, R. J. Heart Lung Transplant 11 (pt. 2): 197 (1992)], adult T-cell leukemia/lymphoma [European Patent Application 525,960 A1], and ocular inflammation [European Patent Application 532,862 A1].

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble aminoacyl prodrugs of rapamycin (U.S. Pat. No. 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions. U.S. Pat. No. 5,023,263 describes the preparation and use of 42-oxorapamycin and U.S. Pat. No. 5,023,264 describes the preparation and use of 27-oximes of rapamycin.

DESCRIPTION OF THE INVENTION

This invention provides derivatives of rapamycin which are useful as immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agents having the structure



wherein X-Y is C=NOR^1 or CHNHOR^2;

R^1 and R^2 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms, alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–77 carbon atoms, fluoroalkyl of 1–6 carbon atoms, trifluoromethylalkyl of 2–77 carbon atoms, trifluoromethyl, ArO—, —$(CH_2)_m$Ar, or —COR_3;

R^3 is alkyl of 1–6 carbon atoms, —NH_2, —NHR^4, —NR^{4}R^5, or —OR_4;

R^4 and R^5 are each, independently, alkyl of 1–6 carbon atoms, Ar or if both are present can be taken together to form a 4–7 membered ring;

Ar is an aryl or heteroaryl radical which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–77 carbon atoms, alkynyl of 2–77 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–77 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–712 carbon atoms, alkylthio of 1–6 carbon atoms, —SO_3H, and —CO_2H; and

m=0–6;

or a pharmaceutically acceptable salt thereof.

The pharmaceutically acceptable salts are those derived from such inorganic cations such as sodium, potassium, and the like; organic bases such as: mono-, di-, and trialkyl amines of 1–6 carbon atoms, per alkyl group and mono-, di-, and trihydroxyalkyl amines of 1–6 carbon atoms per alkyl group, and the like; and organic and inorganic acids as: acetic, lactic, citric, tartaric, succinic, maleic, malonic, gluconic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, and similarly known acceptable acids.

The terms alkyl of 1–6 carbon atoms, alkenyl of 2–77 carbon atoms, and alkynyl of 2–77 carbon atoms, include both straight chain as well as branched carbon chains. By virtue of possessing a double bond, the oximes of this invention possess cis-trans isomerism and hence this invention embraces not only geometrical isomer mixtures, but also the individual E and Z isomers, which can be separated by methods known to those skilled in the art. Likewise, the

hydroxylamines of this invention consist of mixtures of epimers at C-42, and hence this invention embraces not only the isomer mixture, but also the individual isomers, which can be separated by methods known to those skilled in the art.

It is preferred that the aryl and heteroaryl radicals of Ar are phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl that may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–77 carbon atoms, alkynyl of 2–77 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–77 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, —SO_3H, and —CO_2H.

When R^3 is —NR^4R^5, the R^4 and R^5 groups can be the same or different (as defined above) or can be taken together to form a saturated heterocycle having 4–7 atoms in the ring of which 1 atom is a nitrogen and 0–2 other ring atoms can be nitrogen, oxygen, or sulfur. In the case where R^4 and R^5 are taken together, it is preferred that $R4R^5$ is a carbon chain forming an azetidine, pyrrolidine, piperidine, or homopiperidine ring.

Of the compounds of this invention preferred members are those in which X-Y is C=NOR^1; those in which X-Y is C=NOR^1 and R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–77 carbon atoms, alkynyl of 2–77 carbon atoms, alkyloxy of 1–6 carbon atoms, —$(CH_2)_mAr$, or —COR_3; those in which X-Y is $CHNHOR_2$; those in which $CHNHOR^2$, and R^2 is hydrogen or —$(CH_2)_mAr$.

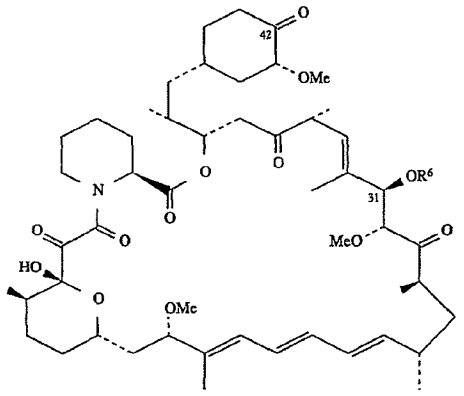
The compounds of this invention can be prepared from 42-oxorapamycin, which can be prepared in moderate yield by selectively oxidizing the 42-position of rapamycin via a ruthenium mediated oxidation as taught in U.S. Pat. No. 5,023,263, which is hereby incorporated by reference. Alternatively, 42-oxorapamycin can be prepared in approximately 50% yield using tetrapropylammonium perruthenate/N-methylmorpholine N-oxide, as taught by Holt in PCT Publication U.S. 93/0668. 42-oxorapamycin can then be treated with an appropriately substituted hydroxylamine to provide mixture of 42-(E) and (Z) oximes (X-Y is C=NOR^1), which can be separated by standard methodology. The 42-oximes can further reacted with a suitable reducing agent, such as sodiron cyanoborohydride/THF/dioxane/pH 3.5, to provide the hydroxylamines of this invention (X-Y is $CHNHOR^2$).

Compounds of this invention in which X-Y is C=NOR^1 and R^1 is a carbonyl containing moiety can be prepared from rapamycin 42-oxime (X-Y is C=NOR^1 and R^1 is hydrogen). For example, rapamycin 42-oxime can be reacted with sodium cyanate to produce the compound in which R^1 is —COR^3 and R^3 is NH_2. Similarly, rapamycin 42-oxime can be treated with a suitable substituted isocyanate or haloacylamine [i.e., $R^4R^5NC(O)Cl$] to provide compounds in which R^1 is—COR^3 and R^3 is—NR^4R^5. Compounds in which R^1 is —COR^3 and R^3 is —NHR^4 can be prepared analogously. Additionally, treatment of rapamycin 42-oxime with an appropriate alkyl- or arylchloroformate in pyridine and a solvent such as methylene chloride provides oxime carbonates in which R^1 is —COR^3, and R^3 is alkyl or Ar.

Analogous functionalization can be obtained when X-Y is $CHNHOR^2$, starting from the compound in which R^2 is hydrogen (obtained by the cyanoborohydride reduction of rapamycin 42-oxime).

As an alternative to the ruthenium based oxidative preparation of 42-oxorapamycin, this invention also provides a synthetic route to the oximes and hydroxylamines of this invention via the Dess-Martin periodinane oxidation of a 31-O-protected rapamycin. The protection of the 31-position of rapamycin has been described in U.S. Pat. No. 5,120,842, which is hereby incorporated by reference. For example, the treatment of rapamycin with a suitable protecting reagent such as triethylsilyl triflate/2,6-utidine/methylene chloride, followed by acetic acid/THF/water provides 31-O-triethylsilyl rapamycin in quantitative yield. Oxidation with Dess-Martin periodinane provides 31-O-triethylsilyl-42-oxorapamycin in about 65% yield, which can then be treated with a suitable hydroxylamine (and subsequently derivatized) as described above. For the oximes of this invention, the triethylsilyl protecting group can be removed under mildly acidic conditions (acetic acid/THF/water) as described in U.S. Pat. No. 5,120,842. Using this route, the hydroxylamines of this invention can also be produced from the corresponding oximes via cyanoborohydride reduction under acidic conditions, with concomitant removal of the silyl protecting group.

Based on the above described Dess-Martin periodinane methodology, the following compounds are intermediates useful in the preparation of the oximes of this invention.



wherein R^6 is —$SiR^7R^8R^9$; and R^7, R^8, and R^9 are each, independently, alkyl of 1–8 carbon atoms, alkenyl of 1–8 carbon atoms, phenylalkyl of 7–10 carbon atoms, triphenylmethyl, or phenyl.

Of the above intermediates, the compound of Example 1 is the preferred intermediate.

The reagents used in the preparation of the compounds of this invention can be either commercially obtained or can be prepared by standard procedures described in the literature.

Immunosuppressive activity for representative compounds of this invention was evaluated in an in vitro standard pharmacological test procedure to measure the inhibition of lymphocyte proliferation (LAF) and in two in vivo standard pharmacological test procedures. The pinch skin graft test procedure measures the immunosuppressive activity of the compound tested as well as the ability of the compound tested to inhibit or treat transplant rejection. The adjuvant arthritis standard pharmacological test procedure measures the ability of the compound tested to inhibit immune mediated inflammation. The adjuvant arthritis test procedure is a standard pharmacological test procedure for rheumatoid arthritis. The procedures for these standard pharmacological test procedures are provided below.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immu-

nosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated radioactivity is determined. Inhibition of lymphoproliferation is assessed as percent change in counts per minute from non-drug treated controls. For each compound evaluated, rapamycin was also evaluated for the purpose of comparison. An IC_{50} was obtained for each test compound as well as for rapamycin. When evaluated as a comparator for the representative compounds of this invention, rapamycin had an IC_{50} ranging from 0.5 to 3.3 nM. The results obtained are provided as an IC_{50} and as the percent inhibition of T-cell proliferation at 0.1 μM. The results obtained for the representative compounds of this invention were also expressed as a ratio compared with rapamycin. A positive ratio indicates immunosuppressive activity. A ratio of greater than 1 indicates that the test compound inhibited thymocyte proliferation to a greater extent than rapamycin. Calculation of the ratio is shown below.

$$\frac{IC_{50} \text{ of Rapamycin}}{IC_{50} \text{ of Test Compound}}$$

Representative compounds of this invention were also evaluated in an in vivo test procedure designed to determine the survival time of pinch skin graft from male BALB/c donors transplanted to male C_3H(H-2K) recipients. The method is adapted from Billingham R. E. and Medawar P. B., J. Exp. Biol. 28:385–402, (1951). Briefly, a pinch skin graft from the donor was grafted on the dorsum of the recipient as a allograft, and an isograft was used as control in the same region. The recipients were treated with either varying concentrations of test compounds intraperitoneally or orally. Rapamycin was used as a test control. Untreated recipients serve as rejection control. The graft was monitored daily and observations were recorded until the graft became dry and formed a blackened scab. This was considered as the rejection day. The mean graft survival time (number of days ±S.D.) of the drug treatment group was compared with the control group. Results are expressed as the mean survival time in days. Untreated (control) pinch skin grafts are usually rejected within 6–7 days. Compounds were tested using a dose of 4 mg/kg, i.p. A survival time of 11.67±0.63 days was obtained for rapamycin at 4 mg/kg, i.p.

The adjuvant arthritis standard pharmacological test procedure measures the ability of test compounds to prevent immune mediated inflammation and inhibit or treat rheumatoid arthritis. The following briefly describes the test procedure used. A group of rats (male inbread Wistar Lewis rats) are pre-treated with the compound to be tested (1 h prior to antigen) and then injected with Freud's Complete Adjuvant (FCA) in the right hind paw to induce arthritis. The rats are then orally dosed on a Monday, Wednesday, Friday schedule from day 0–14 for a total of 7 doses. Both hind paws are measured on days 16, 23, and 30. The difference in paw volume (mL) from day 16 to day 0 is determined and a percent change from control is obtained. The left hind paw (uninjected paw) inflammation is caused by T-cell mediated inflammation and is recorded as % change from control. The right hind paw inflammation, on the other hand, is caused by nonspecific inflammation. Compounds were tested at a dose of 2 mg/kg. The results are expressed as the percent change in the uninjected paw at day 16 versus control; the more negative the percent change, the more potent the compound. Rapamycin provided −70% change versus control, indicating that rapamycin treated rats had 70% less immune induced inflammation than control rats.

The results obtained in these standard pharmacological test procedures are provided following the procedure for making the specific compounds that were tested.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this invention. The results obtained in the LAF test procedure indicates suppression of T-cell proliferation, thereby demonstrating the immunosuppressive activity of the compounds of this invention. Further demonstration of the utility of the compounds of this invention as immunosuppressive agents was shown by the results obtained in the skin graft and adjuvant arthritis standard pharmacological test procedures. Additionally, the results obtained in the skin graft test procedure further demonstrates the ability of the compounds of this invention to treat or inhibit transplantation rejection. The results obtained in the adjuvant arthritis standard pharmacological test procedure further demonstrate the ability of the compounds of this invention to treat or inhibit rheumatoid arthritis.

Based on the results of these standard pharmacological test procedures, the compounds are useful in the treatment or inhibition of transplantation rejection such as kidney, heart, liver, lung, bone marrow, pancreas (islet cells), cornea, small bowel, and skin allografts, and heart valve xenografts; in the treatment or inhibition of graft vs. host disease; in the treatment or inhibition of autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; and diseases of inflammation such as psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease, pulmonary inflammation (including asthma, chronic obstructive pulmonary disease, emphysema, acute respiratory distress syndrome, bronchitis, and the like), and eye uveitis.

Because of the activity profile obtained, the compounds of this invention also are considered to have antitumor, antifungal activities, and antiproliferative activities. The compounds of this invention therefore also useful in treating solid tumors, adult T-cell leukemia/lymphoma, fungal infections, and hyperproliferative vascular diseases such as restenosis and atherosclerosis. When used for restenosis, it is preferred that the compounds of this invention are used to treat restenosis that occurs following an angioplasty procedure. When used for this purpose, the compounds of this invention can be administered prior to the procedure, during the procedure, subsequent to the procedure, or any combination of the above.

When administered for the treatment or inhibition of the above disease states, the compounds of this invention can be administered to a mammal orally, parenterally, intranasally, intrabronchially, transdermally, topically, intravaginally, or rectally.

It is contemplated that when the compounds of this invention are used as an immunosuppressive or antiinflammatory agent, they can be administered in conjunction with one or more other immunoregulatory agents. Such other immunoregulatory agents include, but are not limited to azathioprine, corticosteroids, such as prednisone and methylprednisolone, cyclophosphamide, rapamycin, cyclosporin A, FK-506, OKT-3, and ATG. By combining the compounds of this invention with such other drugs or agents for inducing immunosuppression or treating inflammatory conditions, the lesser amounts of each of the agents are required to achieve the desired effect. The basis for such combination therapy was established by Stepkowski whose results showed that the use of a combination of rapamycin and cyclosporin A at

subtherapeutic doses significantly prolonged heart allograft survival time. [Transplantation Proc. 23: 507 (1991)].

The compounds of this invention can be formulated neat or with a pharmaceutical carder to a mammal in need thereof. The pharmaceutical carder may be solid or liquid. When formulated orally, it has been found that 0.01% Tween 80 in PHOSAL PG-50 (phospholipid concentrate with 1,2-propylene glycol, A. Nattermann & Cie. GmbH) provides an acceptable oral formulation.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carder is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carder can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, lethicins, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which am sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

The compounds of this invention may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insuffiation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. The compounds of this invention may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semi-solid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound which may be administered to a fungally affected area.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily dosages of active compound would be 0.1 μg/kg–100 mg/kg, preferably between 0.001–25 mg/kg, and more preferably between 0.01–5 mg/kg. Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, nasal, or intrabronchial administration will be determined by the administering physician based on experience with the individual subject treated. Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is subdivided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form.

The following examples illustrate the preparation and biological activities of representative compounds of this invention.

EXAMPLE 1

31-O-(Triethylsilyl)-42-oxorapamycin

Step A. 31-O-(Triethylsilyl)rapamycin

To a solution of rapamycin (15.39 g, 16.84 mmole) and 2,6-utidine (7.65 g, 75.76 ml) in dichloromethane (100 ml) at 0° C., triethylsilyl trifluoromethane sulfonate (10 g, 37.88 mmole) was added dropwise over 30 minutes. The mixture was stirred at 0° C. for another 90 minutes, and filtered. The tiltrate was diluted with ethyl acetate (500 ml), washed with water (3×250 ml) and brine (1×100 ml), dried ($MgSO_4$) and evaporated to dryness. The material was redissolved in anhydrous THF (40 ml), cooled to 0° C. and treated with ice-cold glacial acetic acid (150 ml) and water (80 ml). The mixture was stirred for 3 hours at 0° C., diluted with ethyl acetate (500 ml) and carefully brought to pH 7–8 with $NaHCO_3$ at 0° C. The organic layer was washed with water (2×250 ml), brine (1×100 ml), dried ($MgSO_4$) and evaporated to dryness to provide the title product in quantitative yield.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.66 (3H, 6-CH_3C=C), 1.75 (3H, 30-CH_3C=C), 3.14 (3H, 41-OCH_3), 3.27 (3H, 7-CH_3O), 3.40 (3H, 32-CH_3O), 4.12 (m, 1H, 31-CH)

MS (neg. ion FAB, m/z): 1027.4 $[M]^-$, 589.3.

Step B. 31-O-(Triethylsilyl)-42-oxorapamycin

A mixture of 31-O-(triethylsilyl)rapamycin (17.32 g, 16.84 mmole) and Dess-Martin periodinane (8.65 g, 20.35 mmole) in anhydrous dichloromethane (150 ml) was stirred under nitrogen for 5 hours. The mixture was filtered, the tiltrate diluted with ethyl acetate (500 ml) and washed with water (3×250 ml) and brine (1×100 ml), dried ($MgSO_4$) and evaporated to dryness. The crude material was preabsorbed on a silica Merck-60 column and flashed with hexane-ethyl acetate 95:5 and 7:2, to provide pure title product in 64.9% yield.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.42–0.48 (m, 9H), 0.80–0.83 (m, 6H), 1.61 (3H, 6 -CH_3C=C), 1.77 (3H, 30-CH_3C=C), 3.04 (3H, 41-OCH_3), 3.16 (3H, 7-OCH_3), 3.28 (3H, 32-CH_3O), 3.90 (m, 1H, 41-CH).

MS (neg. ion FAB, m/z): 1025.3 $[M]^-$.

EXAMPLE 2

42-Deoxo-42-(hydroxyimino)rapamycin

Preparation A

A mixture of 42-oxorapamycin (0.183 g, 0.22 mmole; prepared via the method described in U.S. Pat. No. 5,023, 263), hydroxylamine hydrochloride (0.0143 g, 0.22 mmole) and sodium acetate (0.025 g, 0.3 mmole) in methanol (5 ml), was stirred under nitrogen for 15 minutes. The mixture was evaporated to dryness and the residue was purified by flash chromatography (on Merck-60 silica gel, eluant 50% THF in hexane). The pure fractions were combined, evaporated and the resulting oil was recrystallized from isopropyl ether/ cyclohexane 20:80 to provide the title product as a mixture of E/Z isomers (0.075 g, 40% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.65 (3H, 6-CH_3C=C)), 1.75 (3H, 30-CH_3C=C), 3.13 (3H, 41-OCH_3), 3.34 (3H, 7-OCH_3), 3.41 (3H, 32-OCH_3) ^{13}C NMR ($CDCl_3$, 400 MHz): δ 215.28, 208.17, 169.26, 166.72, 158.83, 140.60, 140.07, 135.95, 135.67, 133.56, 130.18, 129.47, 126.62, 126.43, 98.48, 86.33, 84,73, 84.30, 78.90, 67.17, 59.29, 59.13, 57.51, 55.93, 55.88, 51.234, 46.58, 46.04, 44.19, 41.47, 40.65, 40.19, 38.93, 38.41, 37.71, 35.08, 33.78, 33.24, 32.05, 32.00, 31.82, 31.21, 30.92, 27.21, 27.05, 26.89, 25.27, 22.84, 21.97, 21.62, 21.46, 20.64, 16.32, 16.22, 16.09, 15.99, 14.74, 13.60, 13.18, 13.10, 10.33, 10.15

MS (neg. ion FAB, m/z): 926 $[M]^-$, 590, 334

Anal. Calcd. for $C_{51}H_{78}N_2O_{13}$: C, 66.07; H, 8.48; N, 3.02; Found: C, 66.25; H, 8.67; N, 3.03.

Preparation B

Step A. 31-O-(Triethylsilyl)-42-deoxo-42-(hydroxyimino)rapamycin

Under anhydrous conditions, a mixture of 31-O-(triethylsilyl)-42-oxorapamycin of Example 1 (0.105 g, 0.102 mmole), hydroxylamine hydrochloride (7.6 mg, 0.109 mmole), and sodium acetate (12.5 mg, 0.153 mmol) in anhydrous methanol (5 ml) was stirred for 30 minutes. The mixture was filtered and the tiltrate evaporated to dryness. The residue was redissolved in ethyl acetate (50 ml), washed with water (2×50 ml) and brine (1×50 ml), dried ($MgSO_4$), and evaporated to dryness to provide the title product as a mixture of E/Z isomers (0.114 g, 94% yield).

^{1}H NMR ($CDCl_3$, 400MHz): δ 0.47–0.55 (m, 9H), 0.84–0.88 (m, 6H), 1.65 (3H, 6-CH_3C=C), 1.75 (3H, 30-CH_3C=C), 3.14 (3H, 41-OCH_3), 3.26 (3H, 7-OCH_3), 3.44 (3H, 32-OCH_3)

MS (neg. ion FAB, m/z): 1040.7 $[M]^-$.

Step B. 42-Deoxo-42-(hydroxyimino)rapamycin

A solution of 31-O-(triethylsilyl)-42-deoxo-42-(hydroxyimino)rapamycin (1 g, 0.974 mmole) in 20 ml of a 10% solution of p-toluenesulfonic acid in methanol, was stirred for one hour under nitrogen at 0° C. The solution was diluted with ethyl acetate and quenched with 5% aqueous $NaHCO_3$. The organic layer was washed with water and brine, dried (MgSO4) and evaporated to dryness to provide the title product, identical with the material described in Preparation A (0.812 g, 89.9% yield).

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 3.88 nM

LAF ratio: 0.85

Skin graft survival: 10.8±0.4

EXAMPLE 3

42-Deoxo-42-(hydroxyamino)rapamycin

To a solution of 31-O-(triethylsilyl)-42-deoxo-42-(hydroxyimino)rapamycin of Example 2, Preparation B, Step A (2.08 g, 2 mmol) in anhydrous methanol (75 ml) under nitrogen and at 0° C., were simultaneously added over a 30 minute period, a 1N-solution of sodium cyanoborohydride in tetrahydrofuran (2 ml) and a 4N HCl solution in dioxane, so as to maintain the pH at 3.5. The mixture was stirred for 30 minutes,, diluted with EtOAc and washed with 2.5% $NaHCO_3$ (100 ml), water (2×250 ml) and brine (1×250 ml), dried ($MgSO_4$), and evaporated to dryness to provide the title compound as a mixture of isomers (0.763 g, 41% yield).

^{1}H NMR ($CDCl_{13}$, 400MHz): δ 1.60 and 1.63 (3H, 6-CH_3C=C), 1.72 and 1.74 (3H, 30-CH_3C=C), 3.09 and 3.11 (3H, 41-OCH3), 3.28, 3.32, 3.34 and 3.36 (6H, 7- and 32-OCH_3)

MS (neg. ion FAB, m/z): 928.4 $[M]^-$, 590.3, 336.6

Anal.: Calcd. for $C_{51}H_{80}N_2O_{13}$: C, 65.92; H, 8.68; N, 3.01; Found: C, 65.36; H, 8.53; N, 2.82.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 5.60 nM

LAF ratio: 0.18

Skin graft survival: 9.6±0.0

Percent change in adjuvant arthritis versus control: –84%

EXAMPLE 4

42-Deoxy-42-oxorapamycin-42-O-carbamoyloxime

A mixture of 42-deoxo-42-(oxyimino)rapamycin of Example 2 (0.813 g, 0.876 mmole), sodium cyanate (0.228 g, 3.5), glacial acetic acid (8 ml) and water (8 ml) was stirred for 1.5 hours under nitrogen. The mixture was diluted with ethyl acetate (100 ml) and quenched with aqueous $NaHCO_3$. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated to dryness. The crude product was dissolved in dichloromethane, preabsorbed on silica gel Merck-60 and purified by flash chromatography (step gradient from 50% ethyl acetate in hexane to pure ethyl acetate) to provide the title product as a mixture of E/Z isomers (0.289 g, 34% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.66 (3H, 6-CH_3C=C), 1.75 (3H, 30-CH_3C=C).

3.14 (3H, 41-OCH_3), 3.33 (3H, 7-OCH_3), 3.43 (3H, 32-OCH_3) ^{13}C NMR (CDC_{13}, 400MHz): d 215.17, 208.11, 169.67, 169.30, 166.73, 164.31, 164.25,

156.37, 156.33, 140.05, 138.34, 137.28, 136.07, 135.78, 135.09, 133.51, 133.25, 132.17, 130.61, 130.22, 130.05, 129.40, 127.33, 126.81, 126.66, 126.57, 126.44, 125.76, 125.50, 125.22, 98.70, 98.50, 84.73, 84.34, 84.28, 82.70, 78.92, 68.91, 67.21, 59.29, 57.93, 57.82, 56.34, 56.31, 56.16, 56.09, 55.85, 55.56, 52.00, 51.25, 46.55, 46.06, 45.68, 44.54, 44.18, 41.92, 41.83, 41.48, 41.40, 41.26, 41.14, 41.01, 40.88, 40.57, 40.42, 40.35, 40.19, 39.77, 39.30, 39.17, 38.97, 38.83, 38.66, 38.59, 37.98, 37.45, 37.26, 35.53, 35.13, 34.88, 34.79, 34.72, 34.57, 33.83, 33.49, 33.37, 33.31, 33.24, 32.19, 32.03, 31.98, 31.85, 21.70, 31.64, 31.20, 27.35, 27.21, 27.03, 26.80, 25.23, 25.04, 24.45, 24.31, 21.62, 21.51, 21.37, 20.99, 20.74, 20.63, 16.69, 16.30, 16.18, 15.95, 15.80, 15.71, 15.03, 14.57, 13.88, 13.64, 13.18, 13.13, 13.07, 10.16

MS (neg. ion FAB, m/z): 969.8 $[M]^-$, 925.8 $[M—CONH_2]^-$, 590.6.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 2.00 nM

LAF ratio: 0.25

Skin graft survival: 9.5±1.1

EXAMPLE 5

42-Deoxy-42-oxorapamycin
42-[O-(pyridin-2-ylmethyl)]-oxime

The title compound was prepared according to Examples 2, Preparation B, except for replacing hydroxylamine hydrochloride with 0-(pyridine-2-ylmethyl) hydroxylamine dihydrochloride (71.1% yield).

^1H NMR ($CDCl_3$, 400MHz): δ 1.654 (3H, 6-CH_3C=C), 1.75 1 (3H, 30-CH_3C=C).

3.138 (3H, 41-OCH_3), 3.334 (3H, 7-OCH_3), 3.338 (3H, 32-OCH_3), 7.17–8.58 (mm, 4H, Harom).

^{13}C NMR ($CDCl_3$, 400MHz): δ 158.96 (42-C=NO—)

MS (neg. ion FAB, m/z): 1017.5 $[M]^-$, 590.4, 425.3.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 1.50 nM

LAF ratio: 0.40

Skin graft survival: 7.8±0.8

Percent change in adjuvant arthritis versus control: –53%

EXAMPLE 6

42-Deoxy-42-oxorapamycin
42-[O-(pyridin-4-ylmethyl)]-oxime

The title compound was prepared according to Example 2, Preparation B, except for replacing hydroxylamine hydrochloride with 0-(pyridine-4-ylmethyl)-hydroxylamine dihydrochloride (34.5% yield).

^1H NMR ($CDCl_3$, 400MHz): δ 1.653 (3H, 6-CH_3C=C), 1.753 (3H, 30-CH_3C=C).

3.153 (3H, 41-OCH_3), 3.29 (2×3H, 7-OCH_3 and 32-OCH_3), 7.2 (m, 2H, Harom), 8.52 (m, 2H, Harom).

MS (neg. ion FAB, m/z): 1017.2 $[M]^-$, 590.2, 425.1.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 1.80 nM

LAF ratio: 0.31

Skin graft survival: 8.0±0.9

EXAMPLE 7

2-Deoxy-42-oxoxapamycin
42-[O-(tert-butyl)]-oxime

The title compound was prepared according to Examples 2, Preparation B, except for replacing hydroxylamine hydrochloride with O-(tert-butyl)-hydroxylamine hydrochloride (25.6% yield).

^1H NMR ($CDCl_3$, 400MHz): δ 1.28 (9H, tert-butyl), 1.654 (3H, 6-CH_3C=C), 1,75 (3H, 30-CH_3C=C), 3.136 (3H, 41-OCH_3), 3.336 (3H, 7-OCH_3), 3.37 (3H, 32-OCH_3)

^3C NMR ($CDCl_3$, 400MHz): δ 155.89 (42-C=NO—)

MS (neg, ion FAB, m/z): 982.5 $[M]^-$, 590.3, 390.2.

Results obtained in standard pharmacological test procedures:

LAF: 49% inhibition at 0.1 μM

EXAMPLE 8

42-Deoxy-42-oxorapamycin
42-[O-(phenylmethyl)]-oxime

The title compound was prepared according to Examples 2, Preparation B, except for replacing hydroxylamine hydrochloride with O-(phenylmethyl)-hydroxylamine hydrochloride (29.9% yield).

^1H NMR ($CDCl_3$, 400 MHz): δ 5 1.652 (3H, 6-CH_3C=C), 1.747 (3H, 30-CH_3C=C).

3.136 (3H, 41-OCH_3), 3.332 (3H, 7-OCH_3), 3.35 (3H, 32-OCH_3), 5.134 (2H, =$NOCH_2$—, at C-42), 7.27–7.37 (m, 5H, Harom)

^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.48 (42-C=NO-)

MS (neg. ion FAB, m/z): 1016.2 $[M]^-$, 590.2, 424.1.

Results obtained in standard pharmacological test procedures:

LAF IC50: 21.67 nM

LAF ratio: 0.03

EXAMPLE 9

42-Deoxy-42-oxorapamycin 42-(O-allyl)-oxime

The title compound was prepared according to Example 2, Preparation B, except for replacing hydroxylamine hydrochloride with O-(allyl)-hydroxylamine hydrochloride (57.1% yield).

^1H NMR ($CDCl_3$, 400 MHz): a 1.655 (3H, 6-CH_3C=C), 1.751 (3H, 30-CH_3C=C).

3.139 (3H, 41-OCH_3), 3.336 (3H, 7-OCH_3), 3.395 (3H, 32-OCH_3), 4.60 (m, 2H,

=$NOCH_2C$=, at C-42), 5.17–5.31 (m, 2H, —C=CH_2, at C-42), 5.94–6.03 (m, 1H,

—CCH=C—, at C-42).

^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.1 (42-C=N-O-)

MS (neg, ion FAB, m/z): 966.5 $[M]^-$, 590.3, 374.2.

Results obtained in standard pharmacological test procedures:

LAF 43% inhibition at 0.1 μM

EXAMPLE 10

42-Deoxy-42-oxorapamycin
42-[O-(prop-2-ynyl)]-oxime

The title compound was prepared according to Example 2, Preparation B, except for replacing hydroxylamine hydrochloride with O-(prop-2-ynyl)-hydroxylamine hydrochloride (35.4% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.66 (3H, 6-CH_3C=C), 1.75 (3H, 30-CH_3C=C).

3.13 (3H, 41-OCH_3), 3.33 (3H, 7-OCH_3), 3.415 (3H, 32-OCH_3), 4.69 (2H, =$NOCH_2$C at C-42)

^{13}C NMR ($CDCl_3$, 400 MHz): δ 159.5 (42-C=NO-)

MS (neg. ion FAB, m/z): 964.2 $[M]^-$, 590.2, 372.1.

Results obtained in standard pharmacological test procedures:

LAF IC50: 8.13 nM

LAF ratio: 0.08

Skin graft survival: 8.0±1.1.

EXAMPLE 11

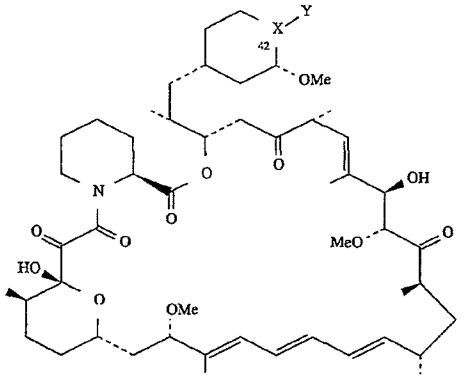
42-Deoxo-42-[O-(pyridin-4-ylmethyl)]-hydroxyamino rapamycin

The title compound was prepared according to Example 3, except for replacing 31-O-(triethylsilyl)-42-deoxo-42-hydroxyimino rapamycin with 42-deoxy-42-oxo rapamycin 42-[O-(pyridin-4-ylmethyl)]-oxime of Example 6.

MS (neg. ion FAB, m/z): 1019.5 $[M]^-$.

What is claimed is:

1. A compound of the structure



wherein X-Y is C=NOR^1;

R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms, alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–7 carbon atoms, fluoroalkyl of 1–6 carbon atoms, trifluoromethylalkyl of 2–7 carbon atoms, trifluoromethyl, ArO—, or —$(CH_2)_m$Ar

Ar is phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, —SO_3H, and —CO_2H; and

m=0–6;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim **1**, wherein R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, alkyloxy of 1–6 carbon atoms, or —$(CH_2)_m$Ar or a pharmaceutically acceptable salt thereof.

3. The compound of claim **1** which is 42-deoxo-42-(hydroxyimino)rapamycin or a pharmaceutically acceptable salt thereof.

4. The compound of claim **1** which is 42-deoxy-42-oxorapamycin 42-[O -(pyridin-2-ylmethyl)]-oxime or a pharmaceutically acceptable salt thereof.

5. The compound of claim **1** which is 42-deoxy-42-oxorapamycin 42-[O -(pyridin-4-ylmethyl)]-oxime or a pharmaceutically acceptable salt thereof.

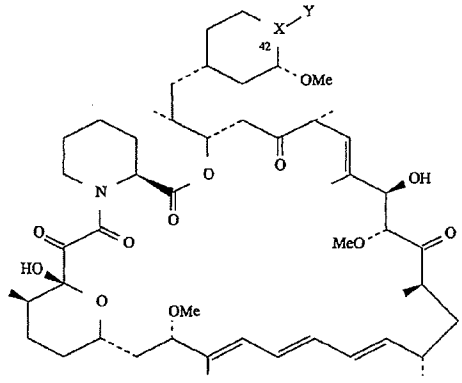
6. The compound of claim **1** which is 42-deoxy-42-oxorapamycin 42-[O-(tert-butyl)]-oxime or a pharmaceutically acceptable salt thereof.

7. The compound of claim **1** which is 42-deoxy-42-oxorapamycin 42-[O-(phenylmethyl)]-oxime or a pharmaceutically acceptable salt thereof.

8. The compound of claim **1** which is 42-deoxy-42-oxorapamycin 42-(O-allyl)-oxime or a pharmaceutically acceptable salt thereof.

9. The compound of claim **1** which is 42-deoxy-42-oxorapamycin 42-[O-(prop-2 -ynyl)]-oxime or a pharmaceutically acceptable salt thereof.

10. A method of treating transplantation rejection or graft vs. host disease in a mammal in need thereof, which comprises administering to said mammal an antirejection effective amount of a compound of the structure



wherein X-Y is C=NOR^1;

R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms,

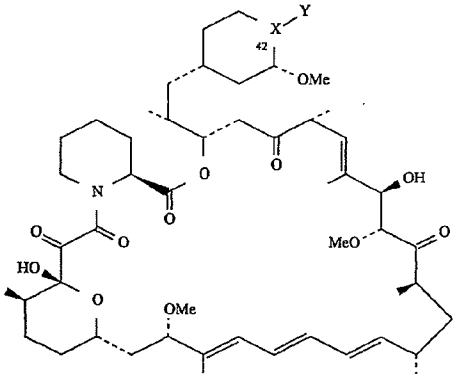
alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–7 carbon atoms, fluoroalkyl of 1–6 carbon atoms, trifluoromethylalkyl of 2–7 carbon atoms, trifluoromethyl, ArO—, or $—(CH_2)_mAr$

Ar is phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, $—SO_3H$, and $—CO_2H$; and

m=0–6;

or a pharmaceutically acceptable salt thereof.

11. A method of treating a fungal infection in a mammal in need thereof, which comprises administering to said mammal an antifungal effective amount of a compound of the structure



wherein X-Y is C=NOR[1];

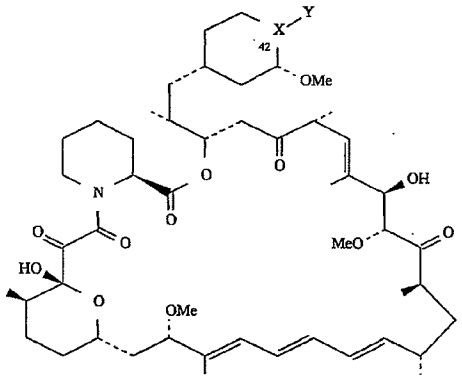
R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms, alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–7 carbon atoms, fluoroalkyl of 1–6 carbon atoms, trifluoromethylalkyl of 2–7 carbon atoms, trifluoromethyl, ArO—, or $—(CH_2)_mAr$

Ar is phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, $—SO_3H$, and $—CO_2H$; and

m=0–6;

or a pharmaceutically acceptable salt thereof.

12. A method of treating rheumatoid arthritis in a mammal in need thereof, which comprises administering to said mammal an antiarthritis effective amount of a compound of the structure



wherein X-Y is C=NOR[1];

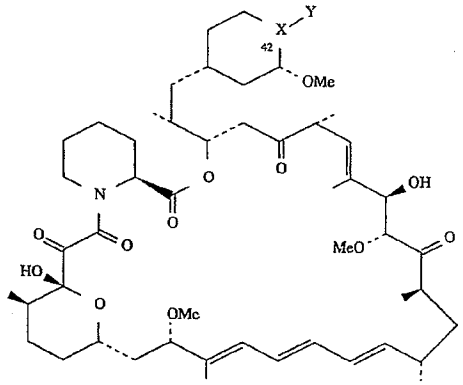
R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms, alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–7 carbon atoms, fluoroalkyl of 1–6 carbon atoms, trifluoromethylalkyl of 2–7 carbon atoms, trifluoromethyl, ArO—, or $—(CH_2)_mAr$

Ar is phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, $—SO_3H$, and $—CO_2H$; and

m=0–6;

or a pharmaceutically acceptable salt thereof.

13. A method of treating restenosis in a mammal in need thereof, which comprises administering to said mammal an antiproliferative effective amount of a compound of the structure



wherein X-Y is C=NOR[1];

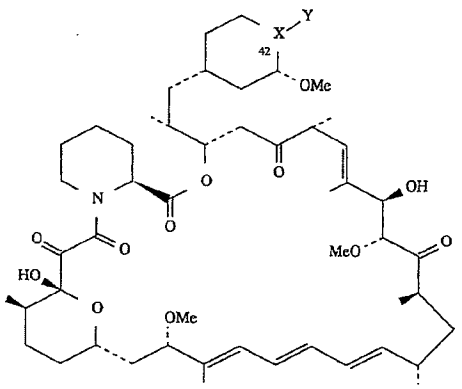
R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms, alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–7 carbon atoms, fluoroalkyl of 1–6 carbon atoms. trifluoromethylalkyl of 2–7 carbon atoms, trifluoromethyl, ArO—, or $—(CH_2)_mAr$

Ar is phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, —SO_3H, and —CO_2H; and

m=0–6;

or a pharmaceutically acceptable salt thereof.

14. A method of treating pulmonary inflammation in a mammal in need thereof, which comprises administering to said mammal an antiinflammatory effective amount of a compound of the structure



wherein X-Y is C=NOR[1];

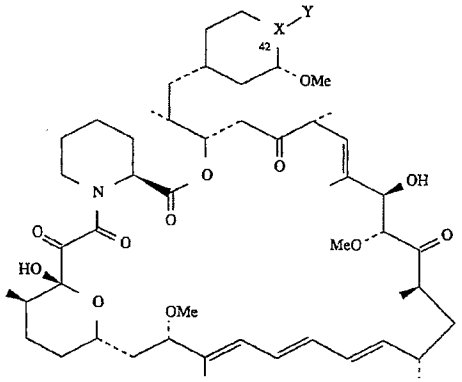
R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms, alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–7 carbon atoms, fluoroalkyl of 1–6 carbon atoms, trifluoromethylalkyl of 2–7 carbon atoms, trifluoromethyl, ArO—, or $—(CH_2)_mAr$

Ar is phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, —SO_3H, and —CO_2H; and

m=0–6;

or a pharmaceutically acceptable salt thereof.

15. A pharmaceutical composition which comprises a compound of the structure



wherein X-Y is C=NOR^1;

R^1 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, aminoalkyl of 1–6 carbon atoms, alkylaminoalkyl of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 1–6 carbon atoms per alkyl group, cycloalkyl of 3–8 carbon atoms, alkyloxy of 1–6 carbon atoms, alkoxyalkyl of 1–6 carbon atoms per alkyl group, cycloalkylaminoalkyl of 4–14 carbon atoms, cyanoalkyl of 2–7 carbon atoms, fluoroalkyl of 1–6 carbon atoms, trifluoromethylalkyl of 2–7 carbon atoms, trifluoromethyl, ArO—, or —$(CH_2)_m$Ar

Ar is phenyl, pyridyl, fury, pyrrolyl, thiophenyl, imidazolyl, oxazolyl, or thiazolyl which may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, hydroxy, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, trifluoromethoxy, amino, dialkylamino of 1–6 carbon atoms per alkyl group, dialkylaminoalkyl of 3–12 carbon atoms, hydroxyalkyl of 1–6 carbon atoms, alkoxyalkyl of 2–12 carbon atoms, alkylthio of 1–6 carbon atoms, —SO_3H, and —CO_2H; and

m=0–6;

or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier.

* * * * *